ANTI BACTERIAL EFFECT OF METHYL PHENYL ACETIC ACID

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Introduction

The fate of ∝-methylphenyl acetic acid has been studied in man and some other mamalian species (Dixon *et al.* 1977a)[1] and it was found to be excreted conjugated with glucuronic acid in all the species examined except the cat which also conjugates it with taurine and glycine. This acid also showed a higher affinity *invitro* to rat microsomal preparation and the associated enzyme than to mitochondria (Dixon et al 1977b)[2].

It is of interest to know the possible spectrum of therapeutic potential of this compound and to examine its antimicrobial activity among others which it might have.

Methodology:

Synthesis :- ∝—methylphenyl acetic acid is prepared by refluxing —methylbenzyl cyanide with a solution of potassium hydroxide in ethanol for 9 hours. The reaction mixture was evaporated to a small volume and acidified with IN HCl and extracted with diethylether. The ethereal layer was then shaken up with saturated solution of sodium bicarbonate and acidified with IN HCl and re-extracted with diethyl ether.

The ethereal layer was dried over anhydrous Magnessium sulphate for 15 hours. The ethereal layer was evaporated in a rotary evaporator leaving a high boiling liquid. The liquid was examined on g. l. c. showed that it contained only a single product and traces of unreacted ∝ —methylbenzyl cyanide. The liquid after distillation left a light yellow high boiling residue, b.p. 262—264°C at 760 mm. ∝ [Lit. value of b.p. of ∝ —methylphenylacetic acid is 264—265°C/760mm ∝ 3.]

The infrared spectrum of the liquid showed a strong carbonyl absorption band at 1720 cm^{-1}. The acid analysed as C4. 5H50 and hence the molecular formula of the compound is $C_9H_{10}0_2$. Its G. C.—Mass spectrum showed a molecular ion at m/e 150. The n.m.r. spectrum contains protons in four different environments in the ratio 1:1:3:5.

Biological Assay:—

The method adopted for the study of the inhibitory effect of ∝—methylphenyl acetic acid on bacterial growth was an agar diffusion method described by Bryant (1972)[4]. However, in our method, we substituted wells for the discs used in Bryant's method. Determination of the bactericidal effect of ∝—methylphenyl acetic acid was carried out after a modified version of the method described by Cruickshank, R.*et al* (1975)[5].

Materials and Method :

Diagnostic sensitivity test agar (DST)
Nutrient agar
Peptone water
Barium sulphate standard solution
Sterile tubes with caps and petri-dishes
Pure cultures of the following bacteria isolated from clinical specimens at the State Hospital, Ile-Ife:

Serratia marcescens
Streptococcus fecalis
Staphylococcus aureus
Escherichia coli
Vibrio cholerae
Shigella sonne
Shigella boydi

Pure cultures of bacteria to be tested were grown on nutrient agar plates at 37°C. About five colonies from each plate were transferred into a tube of peptone water. The peptone water was incubated at 37°C overnight and checked for turbidity the following morning. Heavily turbid growths were diluted with sterile peptone water until the bacterial suspensions were only faintly turbid and comparable with the barium sulphate standard according to the Bauer-kirby technique; Bauer *et al* (1966)[6]. The barium sulphate standard was prepared by adding 0.5 ml of a 1 per cent barium chloride to 99.5 ml. of 1 per cent. $H_2S0_4$0.36 normal. Each inoculum thus compared was estimated to contain about 10^8 bacteria per ml. The bacteria used as inocula were S. marcescens, S. Fecalis, S. aureus, E. coli, V. cholerae, S. sonne, S. boydi and B. subtilis.

Plates of DST and nutriet agar were prepared. Two wells or holes were cut out from each agar plate opposite each other. Each well was 0.5 mm. square.

Inoculation:— Lawn streaking was preferred and used in this method because it gives distinctly clear zones of inhibition and makes reading of the plates easy. Lawn streaking, using each inoculum was carried out on each plate with about 0.15 mls of ∝—methylphenyl acetic acid and the plates were incubated at 37°C for about 24 hours.

The zone of inhibition from the edge of the well to where growth started was measured for each of the two wells on a plate and the average reading was recorded. This was done for all the DST and nutrient agar plates.

Bactericidal Effect of ∝ —methyl phenyl acetic acid:—

Sterile tubes containing 0.5 mls of ∝—methyl phenyl acetic acid were inoculated with bacteria from nutrient agar plates. Each tube was then thoroughly shaken and incubated for 24 hours at 37°C. After 24 hours, the content of each tube was inoculated onto the nutrient agar plate and each plate was incubated at 37°C for about 48 hrs. The plates were then observed for growth.

RESULT

TABLE I

Organism	Zone of inhibition on nutrient agar	Zone of inhibition on DST agar
S. marcescens	1.0 cm	0.9 cm
S. fecalis	1.7 cm	1.5 cm
S. aureus	1.2 cm	1.0 cm
E. Coli	1.2 cm	1.2 cm
V. cholerae	1.0 cm	1.0 cm
S. sonne	1.1 cm	1.0 cm
S. boydi	1.3 cm	1.1 cm
B. subtilis	1.3 cm	0.9 cm

TABLE II

Organism	Growth on nutrient agar after 24 hrs. incubation in α-methyl phenyl acetic acid
S. marcescens	no growth
S. fecalis	no growth
S. aureus	no growth
E. coli	no growth
V. cholerae	no growth
S. sonne	no growth
S. boydi	no growth
B. subtilis	no growth

Discussion :

From the result obtained, all the bacteria tested were sensitive to α-methyl phenyl acetic acid as it inhibited growth in every case. S. fecalis appeared to be the most sensitive bacteria tested as it has the largest zone of growth inhibition while S. marcescens appeared to be the least sensitive of all the bacteria tested having the smallest zone of inhibition. The organisms tested included the gram positive bacteria, the gram negative bacteria, the cocci, bacilli and the vibrio. One can therefore safely infer that α-methyl phenyl acetic acid will inhibit the growth of most bacteria.

The zone of inhibition on the nutrient agar were quite similar to those on the DST agar except in B. subtilis where the zone on nutrient agar 1.3 cm is much larger than that on DST agar 0.9 cm.

Since no growth was obtained from any of the bacteria after incubation with α-methyl phenyl acetic acid, it is clear that the inhibitory effect of α-methyl phenyl acetic acid on bacterial growth is not bacteriostatic but bacteriocidal.

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