Synthesis and study of antibacterial effects of Pentanedial (Glutaraldehyde)

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ABSTRACT

Pentanedial (Glutaraldehyde) is a chemical compound with strong antimicrobial activity which is used as a disinfectant and sterilant agent in operation rooms and laboratories. In this research a novel and simple synthesis of glutaraldehyde was developed and its antimicrobial effects were investigated. Its efficiency was very close to that of commercial ones. The antimicrobial activity of glutaraldehyde was examined in both acidic and alkaline conditions. The results have shown that the 2% alkaline solution of glutaraldehye kills the growth of bacteria (*E. coli, S. typhi, S. epidermidis, P. aeruginosa, S. aureus*) less than a minute, kills viruses (Pollio I, II, III and Herpes simplex), fungi (*Candida albicans* and *Aspergillus niger*) in less than 10 minutes, and kills bacteria spores (*B. subtilis* and *B. anthracis*) in about 7 hours. It was also found that the antimicrobial activity of glutaraldehye in alkaline solution is more effective than in acidic solution, but the alkaline solution lost its activity after two weeks. We also found that addition of divalent ions such as Mg^{2+} to the solution increased the sporocidal activity of glutaraldehyde and reduced the sporocidal time to 3 hours. However, the addition of phenol and sodium phenoxide to the solution not only increased the sporocidal activity of glutaraldehyde and reduced the sporocidal time to 3 hours, but it increased the stability of glutaraldehyde solution to 30 days as well.

INTRODUCTION

Antiseptics and disinfectants are used extensively in hospitals and other health care settings for a variety of topical and hard-surface applications. In particular, they are an essential part of infection control practices and aid in the prevention of nosocomial infections.[1,2] Mounting concerns over the potential for microbial contamination and infection risks in the food and general consumer markets have also led to increased use of antiseptics and disinfectants by the general public. A wide variety of active chemical agents (biocides) are found in these products, many of which have been used for hundreds of years for antisepsis, disinfection, and preservation.[3] Despite this, less is known about the mode of action of these active agents than about antibiotics. In general, biocides have a broader spectrum of activity than antibiotics, and, while antibiotics tend to have specific intracellular targets, biocides may have multiple targets. The widespread use of antiseptic and disinfectant products has prompted some speculation on the development of microbial resistance in particular cross-resistance to antibiotics.[4] Considerable progress has been made in understanding the mechanisms of the antibacterial action of antiseptics and disinfectants.[5,6,7] By contrast, studies on their modes of action against fungi[8,9], viruses[10,11] and protozoa[12]

have been rather sparse. Whatever the type of microbial cell (or entity), it is probable that there is a common sequence of events. This can be envisaged as interaction of the antiseptic or disinfectant with the cell surface followed by penetration into the cell and action at the target site(s). The nature and composition of the surface vary from one cell type (or entity) to another but can also alter as a result of changes in the environment.[13,14] Interaction at the cell surface can produce a significant effect on viability (e.g. with glutaraldehyde)[15,16], but most antimicrobial agents appear to be active intracellularly.[17,18] The outermost layers of microbial cells can thus have a significant effect on their susceptibility (or insusceptibility) to antiseptics and disinfectants; it is disappointing how little is known about the passage of these antimicrobial agents into different types of microorganisms. Glutaraldehyde is an important dialdehyde that has found usage as a disinfectant and sterilant, in particular for low-temperature disinfection and sterilization of endoscopes and surgical equipment and as a fixative in electron icroscopy. Glutaraldehyde has a broad spectrum of activity against bacteria and their spores, fungi, and viruses. The first reports in 1964 and 1965 demonstrated that glutaraldehyde possessed high antimicrobial activity. Subsequently, research was undertaken to evaluate the nature of its bactericidal and sporicidal action. These bactericidal studies demonstrated a strong binding of glutaraldehyde to outer layers of organisms such as *E. coli* and *Staphylococcus aureus* inhibition of transport in gram-negative bacteria, inhibition of dehydrogenase activity and of periplasmic enzymes, prevention of lysostaphin-induced lysis in *S. aureus* and of sodium lauryl sulfate-induced lysis in *E. coli,* inhibition of spheroplast and protoplast lysis in hypotonic media, and inhibition of RNA, DNA, and protein synthesis. Strong interaction of glutaraldehyde with lysine and other amino acids has been demonstrated.[4]

EXPERIMENTAL

Reagents

All reagents and chemicals were of analytical reagent grade and were purchased from Merck. They were used without further purification for the preparation and synthesis of the intermediates and products.

Apparatus

All intermediates and products were routinely examined by proton NMR (Bruker, Germany, 400 MHz) and (Hitachi R60, Japan, 60 MHz), IR (JASCO, 700 IR) and mass spectrometer (Finnigan MAT: Q70, USA).

1.   Preparation of 1,3-dibromopropane (II)

In a 1000 mL round-bottomed flask, 500 g (338 mL) of 48% hydrobromic acid was placed and 150 g (82 mL) concentrated sulphuric acid was added in portions with shaking. Then, 1,3-propanediol (91 g, 1.197 mol) (b.p. 210-215°C, n_D=1.4398) was added followed by gradual addition of concentrated sulphuric acid (240 g, 130.5 mL) with shaking. The mixture was refluxed for 4-6 hours, then, distilled. The distillation process continued until no more oily drops passed over (about 60-80 minutes). The distillate was transferred into a separatory funnel. The lower layer (the desired one) was separated and washed successively with equal volumes of water, concentrated hydrochloric acid, 5% aqueous solution of sodium bicarbonate and finally with water. The organic layer was separated and dried over anhydrous magnisum sulphate. Filtration, followed by distillation afforded 1,3-dibromopropane (218 g, 1.08 mol, 90%). Its b.p. was 165-167°C.[19,20] Its IR spectrum (neat liquid) showed ν^-(cm^{-1}): 3000-2818 (CH, s), 1431, 1417 (CH$_{2, bending}$, m), 650-540 (C-Br, m); its HNMR (CDCl$_3$, 60 MHz) showed (δ): 2.9 (quintet, 2H), 4.2 (t, 4H).

2.   Preparation of pentanedinitrile (III)

Finely powdered sodium cyanide (147 g, 3mol) and 150 mL water were placed in a 2000 mL two-necked round-bottomed flask equipped with a separatory funnel and a reflux condenser. The flask was heated on a water bath until most of the solid passed into solution. 1,3-dibromopropane (250 g, 1.24 mol) was dissolved in absolute ethanol (500 mL) and transferred into the separatory funnel then, added dropwise to the solution inside the flask over a period of 45 minutes. The mixture was refluxed on a water bath for 40 hours, then, the solvent was removed on a rotary evaporator. The residue in the flask consisted of sodium bromide, unreacted

sodium cyanide and the desired product i.e. dinitrile. The residue was extracted with ethyl acetate (3 x 70 mL). The organic layers were combined and filtered through a sintered glass funnel. The solid was washed with 50 mL ethyl acetate. The filtrate was dried over calcium sulphate. Filtration followed by removal of ethyl acetate at atmospheric pressure (about 245 mL), then distillation of the remained liquid at reduced pressure (144-147 °C/13 mmHg), afforded pentanedinitrile (90 g, 0.957 mol, 77%). Its IR spectrum (neat liquid) showed ν^-(cm^{-1}): 2950 (CH, s), 2248 (CN, m), 1454, 1426 (CH$_{2, bending}$, m); its HNMR (CDCl$_3$, 60 MHz) showed (ν): 2.4 (quintet, 2H), 2.9 (t, 4H).

3. Preparation of pentanedial (glutaraldehyde) (IV)

In a 1000 mL three-necked round-bottemed flask equipped with a mechanical stirrer, a reflux condenser and a gas tube, 57 g anhydrous stannous chloride and 200 mL dry ethyl ether were placed. Then, dry hydrogen chloride gas was passed into the mixture until saturation and the layers separated from each other. The sticky lower layer contained stannous chloride which was dissolved in the ethereal hydrogen chloride (it took 2 hours). The gas tube was replaced by a separatory funnel which contained pentanedinitrile (III) (9.4 g, 0.1 mol). While the mixture was stirred, pentanedinitrile was
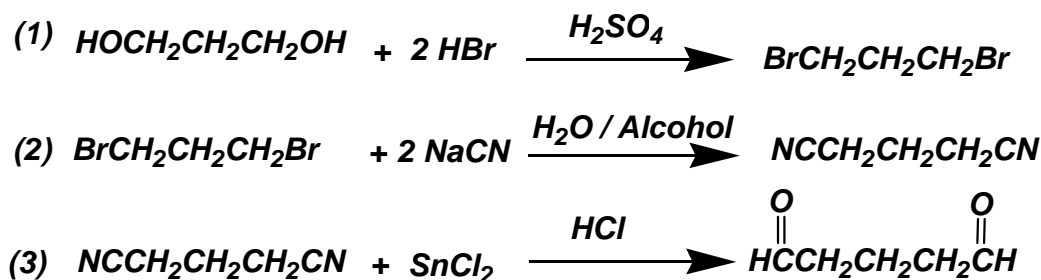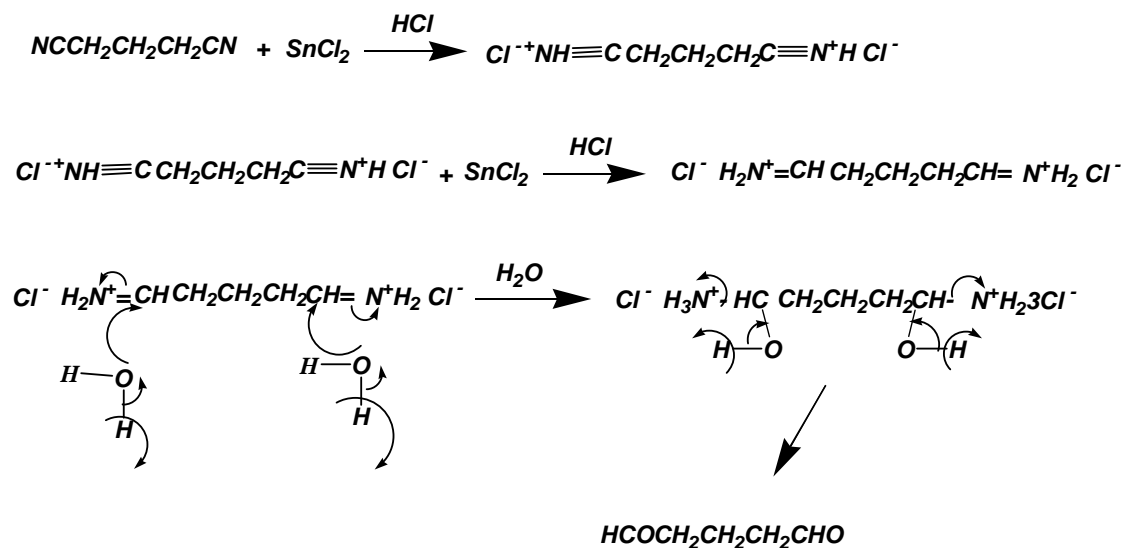

Fig. - 1: synthesis of pentanedial (glutaraldehyde)

Scheme - 1: Mechanism of formation of glutaraldehye

added from the separatory funnel. Stirring was continued for 5 hours. The crystalline material was collected over a filter paper then, poured into 50 mL distilled water and stirred until a suspension was formed. The suspension was transferred into a 100 mL round bottomed flask and refluxed on a water bath until it was dissolved completely. The mixture was cooled and glutaraldehyde was extracted with ether. The ethereal solution was dried over anhydrous sodium sulphate. Ether was removed by distillation and pentanedial (glutaraldehyde) (6.5 g, 0.065 mol, 65%) was collected at 71-72°C/ 10 mmHg. Its IR spectrum (neat liquid) showed ν'(cm^{-1}): 3000-2800 (CH, s), 2700 (CHO, m), 1725 (C=O, s), 1450-1300, (CH$_{2, bending}$, m); its HNMR (CDCl$_3$, 400 MHz) showed (δ): 1.96 (quintet, 2H), 2.55 (t, 4H), 9.78 (s, 2H); its MS (EI) showed m/z: 101 {[M+1]$^{+}$·, 65%}, 101 {[M+1]$^{+}$·, 65%}, 100 {[M+]$^{+}$·, 2%}, 82 {[M-H$_2$O]$^{+}$·, 66%}, 72 {[M-CO]$^{+}$, 58%}, 57 {[M- CH$_2$ CHO]$^{+}$, 65%}, 44 {[M-2CO]$^{+}$, 100%}, 43{[M- CH$_2$CH$_2$ CHO]$^{+}$, 62%}.

4. Antibactorial, antimicrobial and fungicidal characteristic tests

All these extensive and comprehensive tests were carried out by using the synthesized glutaraldhye in this research on bacteria (*E. coli, S. typhi, S. epidermidis, P. aeruginosa, S. aureus*), viruses (Pollio I, II,III and Herpes simplex), fungi (*Candida albicans* and *Aspergillus niger*) and bacteria spores (*B. subtilis* and *B. anthracis*) by our colleagues at the department of Microbiology, School of Basic Medicines and the results will appear in a relevant Journal in due time. Just the final results are given briefly in this paper.

DISCUSSION

Low concentrations of both acidic and alkaline glutaraldehyde increase the surface hydrophobicity of spores, indicating an effect at the outermost regions of the cell. It is conceivable that acidic glutaraldehyde interacts with and remains at the cell surface whereas alkaline glutaraldehyde penetrates more deeply into the spore. Novel glutaraldehyde formulations based on acidic rather than alkaline glutaraldehyde, which benefit from the greater inherent stability of the aldehyde at lower pH, have been produced. The improved sporicidal

activity claimed for these products may be obtained by agents that potentiate the activity of the dialdehyde.[21,22] Clearly, the mechanism of action of glutaraldehyde involves a strong association with the outer layers of bacterial cells, specifically with unprotonated amines on the cell surface, possibly representing the reactive sites.[23]Glutaraldehyde is more active at alkaline than at acidic pHs. As the external pH is altered from acidic to alkaline, more reactive sites will be formed at the cell surface leading to a more rapid bactericidal effect. The cross-links thus obtained mean that the cell is then unable to undertake most, if not all, of its essential functions. Pentanedial (Glutaraldehyde) is a chemical compound with strong antimicrobial activity which is used as a disinfectant and sterilant agent in operation rooms and laboratories. Glutaraldehyde is produced by different chemical companies, but since the chemicals which are used in the synthesis of glutaraldehyde by various commercial companies are not easily available, therefore, in this research a novel and simple synthesis of glutaraldehyde was developed and its antimicrobial, antivirus, antifungal and antibacterial spores effects were investigated. In this synthetic strategy (Fig. -1 and Scheme -1), first, 1,3-propanediol **(I)** was converted into the corresponding 1,3-dibromopropane **(II)** by treating it with concentrated hydrobromic acid. Then, 1,3-dibromopropane was treated with sodium cyanide to produce 1,3-dicyanopropane **(III)**. Hydrolysis of the latter by stannous chloride and hydrochloric acid afforded glutaraldehyde **(IV)**.The antimicrobial activity of glutaraldehyde was examined in both acidic and alkaline conditions. The extensive results obtained from these studies by our colleagues at the Microbiology department which are beyond the scope of this article, showed that the efficiency of the synthesized glutaraldehyde was very close to that of the commercial ones. The results have shown that the 2% alkaline solution of glutaraldehye kills the growth of bacteria (*E. coli, S. typhi, S. epidermidis, P. aeruginosa, S. aureus*) in less than a minute, kills virus (Pollio and Herpes) and fungi (*Candida* and *Aspergillus*) in less than 10 minutes, and kills bacteria spores (B. subtilis and B. anthracis) in about 7 hours. We also found that the antimicrobial activity of glutaraldehye in alkaline solution is more effective than in acidic solution,

but the alkaline solution lost its activity after two weeks. It was also found that addition of divalent ions such as Mg^{2+} to the solution increased the sporocidal activity of glutaraldehyde and reduced the sporocidal time to 3 hours. However, the addition of phenol and sodium phenoxide (sodium phenate/phenolate) to the solution not only increased the sporocidal activity of glutaraldehyde and reduced the sporocidal time to 3 hours, but it also increased the stability of glutaraldehyde solution to 30 days.

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