SYNTHESIS, CHARACTERISATION AND ANTIBACTERIAL EVALUATION OF 2(5H)-FURANONE DERIVATIVES FROM HIGHLY FUNCTIONALISED MUCOBROMIC ACID

C. V. SINDHU RAMACHANDRAN *, P. K. SREEKUMAR1

1Department of Chemistry, NSS College, Manjeri, Malappuram, Calicut University, Kerala State, India, 2Department of Chemistry, NSS College, Pandalam, Pathanamthitta, Kerala University, Kerala State, India. Email: sinducy@gmail.com

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ABSTRACT

Mucobromic acid or 3,4-dibromo-5-hydroxy-2(5H)-furanone is a highly functionalised, inexpensive starting material for the synthesis of furanone derivatives. Starting from mucobromic acid, new derivatives were prepared and characterised. Mucobromic acid and its new derivatives were tested for their antibacterial activity against Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa and Staphylococcus aureus.

Keyword: Mucolic acid, 3, 4-dibromo-5-hydroxy-2(5H)-furanone, 2,3-dibromo-3-formyl Acrylic acid, 2,3-Dibromo-4-Oxo-2-Butenoic Acid. Antibacterial studies

INTRODUCTION

2(5H)-Furanone derivatives are a large family of heterocycles that include synthetically useful compounds, several natural products and drugs with diverse biological activities. The unsaturated lactones are able to inhibit selectively both tissue growth and seed germination. These applications illustrate that these mucolic acids can provide a simple and convenient entry to a wide variety of interesting organic compounds. Nevertheless, our attempt is concerned on the use of mucobromic acid for preparation of unsaturated halogenated derivatives, which are precursors to biologically and agro chemically important substances.

Mucolic acids were first investigated by Hill and Simonis from 1850-1905. Mucobromic acid or 3,4-dibromo-5-hydroxy-2(5H)-furanone is a highly functionalised, inexpensive starting material for synthesis of variety of organic compounds. Compounds containing the lactone functions have exhibited a broad range of physiological properties including antitumour, antibiotic, haemorrhagic and insecticidal activity. Reactions of mucolic acid series with di- thionylamine and its methyl ester gave the fused γ-lactam thiazolidines which are structurally related to penicillin's.

Mucobromic acid was prepared from furanic acid by treatment with bromine in 60-70% yield or it may be prepared from furfural with bromine. To study the reactivity of the system towards nucleophiles, the first series of reaction was carried out with hydrazine and its derivatives and it was found that pyridazone derivatives were the products. Starting from mucobromic acid, pseudo ester 3, 4-dibromo-5-benzoyloxycrotonolactone was prepared by the procedure adopted from Mowry and David. The studies in these fields have been summarised and discussed in several excellent reviews, some of which have been recently published.

Stable reaction products of mucolic acid with aromatic and heterocyclic thiols were synthesized and characterized. Under basic conditions the reactions proceed with the substitution of the chlorine atom(s) by arylthio group(s), while in an acidic medium the hydroxyl group at C2 was substituted. Different types of new sulphur-containing products of di- and tri substitution on the basis of mucobromic acid were obtained. In one case a new acyclic product di-p-tolyl-2,3-bis(p-tolythio)butane dithioate was isolated. The structure of all synthesized compounds was confirmed by IR, 1H and 13C NMR spectroscopy; three compounds were characterised by single crystal X-ray diffraction.

MATERIALS AND METHODS

Analar grade and commercially available media were purchased from BDH, Glaxo and E-Merck. UV spectra were recorded with a shimadzu160-A spectrometer. IR spectra were recorded on a Schimadzu DR8001 FT-IR. PMR spectra were recorded at 400MHz on a Jeol GSX 400NB FT-NMR. Mass spectra were obtained on a Finnigan Mat 8230 spectrometer.

3,4-Dibromo-5-ethoxycrotonolactone (I)

Mucobromic acid (20g, 0.076mol) and absolute ethanol (50mL) were refluxed for 8h in presence of concentrated H2SO4(0.2mL) on a water bath. Excess solvent was removed by distillation and the residue was diluted with water and extracted with ether. The ether layer was washed with saturated NaHCO3 solution and dried using sodium sulphate. On removal of solvent a lachrymotic solid is obtained. mp. 50°C. UV (EtOH) λmax (ε) 243 nm (8700):

IR (KBr); 1215cm−1 (C=O), 1610cm−1 (C=C), 1790cm−1 (C=O)

PMR; (CDCl3) δ 1.25 (t, 3H), δ 3.8(q, 2H), δ 5.82(s, 1H)

5-Benzoyloxy-3,4-dibromocrotonolactone (II)

Mucobromic acid (25.8g, 0.1mol) was refluxed with benzoyl chloride (14.1g, 0.1mol) in a flask fitted with condenser attached with a tube which was dipped in 2N NaOH solution. The mixture was refluxed until the evolution of HCl ceased. The resulting solid was washed with hexane and recrystallised from ethyl acetate-hexane to give 23.2g (90%) of white solid, mp 153°C.

UV (EtOH); λmax (ε) 240nm (20170): 279nm (1300)

IR (KBr); 1240, 1260cm−1 (C=O), 1610cm−1 (C=O), 1750cm−1 (ester C=O), 1800 cm−1 (lactone C=O)

PMR; (CDCl3) δ 7.28(s.1H): δ 7.68‐8.16 (m, 5H)


3,4-Dibromocrotonolactone (III)

To a mixture of mucobromic acid (1g, 0.0039mol) in water (10mL) was added NaBH4(0.15g, 0.0018mol) with stirring. After stirring for 1h at room temperature it was diluted with 2N HCl and cooled. The solid was collected and recrystallised from hexane to give 65% product. Mp. 90°C.

UV (CH3OH); λmax (ε) 237 nm (7000):

IR (KBr); 1215cm−1 (C=O), 1610cm−1 (C=O), 1790cm−1 (C=O)

PMR; (CDCl3) δ 4.92(s.2H):

MS; m/z 240(M+)}

5-Chloro 3,4-dibromocrotonolactone (IV)

A mixture of mucobromic acid (17.7g, 0.066mol), thionyl chloride (50mL) and 2drops of DMF was heated to 50°C for 5h on a water bath. Excess thionyl chloride was removed under vacuum and...
remaining solid washed with water. The solid was dissolved in alcohol and water was added to initiate crystallization, the mixture was cooled to get 52% of white lachrymal solid. M.p 49°C

UV (EtOH); λmax (e ) 247 nm (9700)
IR (KBr); 1600 cm⁻¹ (C=O), 1700 cm⁻¹ (C=C), 3250 cm⁻¹ (OH)

PMR (DMSO); δ 5.88 (s, 1H), δ 3.89 (q, 2H), δ 1.34 (t, 3H); MS; 247

alcohol and water was added to initiate crystallization, the mixture was washed with sodium thiosulphate, decolourised with acetonitrile was added and stirred for 24h at room temperature. The residue diluted with water and extracted with methylene chloride was refluxed in methanol for 3h. Excess solvent was removed and then with water. The solvent was dried and evaporated to give 44.32% white crystalline product. M.p 70°C

IR (KBr) ;1220 cm⁻¹ (C=O), 1640 cm⁻¹ (C=C), 1790 cm⁻¹ (C=O), 2120 cm⁻¹ (N=H)

Anal: calculated for C₄HBr₂ClO₂ : C, 17.38; H,0.36. Found: C, 17.58; H, 1.36; N, 8.34.

RESULTS

Mucobromic acids, which are γ aldehydic acids, can have the 3, 4-dibromo-5-hydroxycrotonolactone structure (1) or α, β-formyl structure (2) and under favorable conditions react as either tautomeric form. The UV spectra of mucobromic acid in ethanol showed a strong λ max at 242nm and another at 275nm. In 0.1N alkali, the absorption at 242nm disappears, while a single band at 275nm remains. This observation correlated with penicillic acid which showed 225nm in aqueous solution due to pseudo acid form and shifted to 295nm in 0.1N alkali.¹¹

4-Amino-3-bromo-5-benzoyloxytocrotonlactone(I)

A mixture of 5-Benzoxyl-3,4-dibromocrotonlactone(1.6g, 0.0045mol) and sodium azide (0.6g, 0.009mol)was refluxed in acetonitrile for 4h resulting in a dark viscous red oil. This was dissolved in 50ml water and extracted with 200ml ether. The ether layer on evaporation gave light yellow solid, which on recrystallisation from 50% ethanol gave a white solid (45%) m.p 204-206°C

UV (95%EtOH); λmax (e ) 269 nm (16400), 235nm(14900)
IR (KBr) ;1610 cm⁻¹ (C=O), 1730 cm⁻¹ (ester C=O), 1775cm⁻¹ (lactone C=O), 3410 and 3225

MS; 229 (M+)

Anal: calculated for C₄HBr₂ClO₂ : C, 17.38; H,0.36. Found: C, 17.58; H, 1.36; N, 8.34.

Fig. 1: Tautomeric forms of Mucobromic acid

The ester derivatives of the acids were suggested by Hill to be in the cyclic or pseudo structure 12. 5-Ethoxy-3,4-dibromocrotonolactone(I) or pseudo ester of mucobromic acid was prepared by refluxing mucobromic acid with absolute ethanol in presence of conc. sulphuric acid for 8h. The ethoxy ester showed UV absorption at 243nm characteristic of the pseudo form of the acid.

Mucobromic acid on refluxing with benzoyl chloride gave a solid with m.p.155 °C, which was characterized as 3,4-dibromo-5-benzoyloxytocrotonlactone(II) by spectral and analytical methods. Mucobromic acid was reduced with NaBH₄ in water at 0°C and was characterized as 3,4-dibromo-5-benzoyloxytocrotonlactone(II) by spectral and analytical methods. Mucobromic acid was reduced with NaBH₄ in water at 0°C and was characterized as 3,4-dibromo-5-benzoyloxytocrotonlactone(II) by spectral and analytical methods. Mucobromic acid was reduced with NaBH₄ in water at 0°C and was characterized as 3,4-dibromo-5-benzoyloxytocrotonlactone(II) by spectral and analytical methods.
carbonyl group followed by elimination of Br on the same carbon atom. To identify the position of azide group on mucobromic acid, the same reaction was repeated with derivatives of mucobromic acid. Since the pseudoester could give only the β substituted product, the product was identified as 4-azido-3-bromo-5-ethyxcrotonolactone (VII). IR spectra of VII showed strong azide absorption, unsaturation and carbonyl peaks. UV spectra of VII showed intense absorption at 274nm where as in pseudo ester absorption was at 243nm. The red shift to 274nm may be due extended conjugation of azide group at the β position. The azide substituted mucobromic acid also showed UV max at 274nm compared to 242nm in the acid, suggesting the substitution is on the β position. Thus the azide substitution in mucobromic acid is in the 4th position or at β position of the carbonyl group which is verified by the NMR data. Mucobromic acid gave NMR signal at 5.7 δ in CD3CN, which is attributed by the proton carbonyl group which is verified by the NMR data. Mucobromic acid is in the 4th position or at β position of the carbonyl group.

Antibacterial Studies

The micro organisms used were supplied from the stock collections of Department of Biotechnology, University of Kerala, Thiruvananthapuram. Antibacterial activity of compounds were determined by the micro dilution method according to the National Committee for Clinical Laboratory Standards (NCCLS) and paper disc diffusion technique. The bacterial strains used for the study are, three-Gram negative organisms (Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa) and one gram positive organism (Staphylococcus aureus). Stock solutions of the compounds were prepared in DMSO. These samples were applied to paper disc having 5mm diameter (Whatman No:1) with the help of a micropipette. The disc was kept in an incubator for 24 hours at 37°C. Commercially available standard Gentamycin discs were used as a standard antibiotic against all the bacterial strains studied.

In order to clarify any participating role of the solvent, DMSO in the antibacterial screening, separate studies were carried out with DMSO as solvent control and it showed no meaningful activity against the bacterial strains under study. The activities of compounds were also compared with a known antibiotic, gentamycin (10μg disc⁻¹).

For the standard drug, the exhibited inhibition zone diameter was in the range of 15 – 25mm against all the bacterial strains used in this study. All the compounds showed comparable activity against the pathogens. The diameter of inhibition zones for various samples are shown in the table I. Compounds I and V showed more activity than other three compounds against the bacterial strains.

Antibacterial screening also showed compounds are more sensitive towards Gram negative bacteria such as Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa. Therefore it is claimed here that such compounds might have a possible antitumour effects since Gram negative bacteria are considered a quantitative microbiological method for testing beneficial and important drugs in both clinical and experimental tumour chemotherapy.
Table 1: Antibacterial activity of Mucobromic acid and its derivatives

<table>
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<th>Bacterial strains</th>
<th>MBA</th>
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REFERENCES