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Research Article

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

C.V. SINDHU RAMACHANDRAN *, P.K SREEKUMAR[1]

*[1]Department of Chemistry, NSS College, Manjeri, Malappuram, Calicut University, Kerala State, India, [2]Department of Chemistry, NSS College, Pandalam, Pathanamthitta, Kerala University, Kerala State, India. Email: sinducv@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: Mucohalic 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 mucohalic 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.

Mucohalic 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[1,2]. Reactions of mucohalic acid series with dl penicillamine and its methyl ester gave the fused γ-lactam thiazolidines which are structurally related to penicillin's[3].

Mucobromic acid was prepared from furanoic acid by treatment with bromine in 60-70% yield or it may be prepared from furfural with bromine [4,5]. 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[6]. Starting from mucobromic acid, pseudo ester 3, 4-dibromo-5-benzoyloxycrotonolactone was prepared by the procedure adopted from Mowry and David.[7] The studies in these fields have been summarised and discussed in several excellent reviews, some of which have been recently published [8]. Stable reaction products of mucohalic acid with aromatic and heterocyclic thiols were synthesized and characterized. Under basic conditions the reactions proceeded with the substitution of the chlorine atom(s) by arylthiogroup(s), while in an acidic medium the hydroxyl group at C_5 was substituted. Different types of new sulphur-containing products of di and tri substitution on the basis of mucochloric acid were obtained. In one case a new acyclic product di-p-tolyl-2,3-bis-(p-tolylthio)butane dithioate was isolated. The structure of all synthesized compounds was confirmed by IR, ^{1}H, and ^{13}CNMR spectroscopy; three compounds were characterized by single crystal X-ray diffraction[9].

MATERIALS AND METHODS

Analar grade chemicals 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 H_2SO_4 (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 $NaHCO_3$ solution and dried using sodium sulphate. On removal of solvent a lachrymatric solid is obtained.mp. 50°C [10].

UV (EtOH) λ_{max} (ε) 243 nm (8700):

IR (KBr) 1210cm-1 (C-O), 1610cm-1 (C=C), 1789cm-1 (C=O)

PMR (DMSO) 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.m.p 153°C.

UV (EtOH); $\lambda_{max;}$(ε)240nm (20170): 279nm(1300)

IR (KBr); 1240, 1260cm^{-1} (C-O), 1610cm-1 (C=C), 1750cm^{-1} (ester C=O), 1800 cm-1(lactone C=O)

PMR; ($CDCl_3$) δ 7.28(s.1H): δ 7.68-8.16 (m, 5H)

Anal: calculated for $C_{11}H_8Br_2O_4$: C, 36.49: H,1.67. Found: C, 36.77: H, 1.80.

3,4-Dibromocrotonolactone(III)

To a mixture of mucobromic acid (1g, 0,0039mol) in water (10mL) was added $NaBH_4$ (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. M.p. 90°C.

UV (CH_3OH) ;λ_{max}(ε) 237 nm (7000):

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

PMR;($CDCl_3$) δ 4.92(s.2H):

MS; m/z 240(M+)

5-Chloro 3,4-dibromocrotonolactone(IV)

A mixture of mucobromic acid (17.7g, 0.006mol), 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^{o}C

UV (EtOH); $\lambda_{max;}$ (ε) 247 nm (9700)

IR (KBr) ;1200cm^{-1} (C-O), 1600cm^{-1} (C=C), 1780cm^{-1} (C=O)

PMR ($CDCl_3$); δ 6.6(s, 1H),

Anal: calculated for $C_4HBr_2ClO_2$: C, 17.38 : H,0.36. Found: C, 17.58: H, 0.43.

1-Carboxamido-3,4-dibromo-5-hydroxy-3-pyrroline-2-one(V)

Mucobromic acid(100mg, 0.0003mol) and urea (200mg, 0.003mol) was refluxed in methanol for 3h. Excess solvent was removed and residue diluted with water and extracted with methylene chloride (50mL).The product was purified on silica column using $CHCl_3$-CH_3OH(99:1)as eluent to give 65% product.

M.p 170^{0}C

UV (CH_3OH); $\lambda_{max;}$ (ε) 240 nm (15150), 203nm(9090)

IR (KBr) ;1614 cm-1 (C=C), 1700cm-1 (lactone C=O), 3250, 3372 and3470cm^{-1}(NH_2 , OH)

PMR(DMSO); δ 6.2 (s,2H), δ 6.5(d,1H), δ7.5(d,1H)

Anal: calculated for $C_5H_4Br_2N_2O_3$: C,20.13: H,1.34: N,8.34. Found: C,19.09: H,1.36: N,8.34.

4-Azido-3-bromo-5-hydroxycrotonolactone(VI)

A mixture of sodium azide (6.5g, 0.108mol) and acetonitrile (40mL) was cooled to 0^{o}C. To this mucobromic acid (14g, 0.054mol) in 20mL acetonitrile was added and stirred for 24h at room temperature. The mixture was diluted with water, extracted with ether. The ether layer was washed with sodium thiosulphate, decolourised with carbon and evaporated to dryness under reduced pressure to give 72% white crystalline product. Mp 70^{o}C

UV (CH_3OH); $\lambda_{max;}$ (ε)274 nm (18500):

IR (KBr) ;1220cm^{-1} (C-O), 1625cm^{-1} (C=C), 1760cm^{-1} (C=O), 2120 cm^{-1}(N_3), 3250 cm^{-1}(OH)

PMR(DMSO); δ 4.53 (s, 1H), δ 6.67(s, 1H)

4-Azido-3-bromo-5-ethoxycrotonolactone(VII)

To an ice cold solution of sodium azide (0.9g, 0.014mol) in 10mL acetonitrile was added 3,4-dibromo-5-ethoxycrotonolactone in 10mL acetonitrile very slowly . The reaction mixture was stirred for 48 h . It is then diluted with water and extracted with chloroform to get a low melting solid (70%).

UV (EtOH); $\lambda_{max;}$ (ε) 274 nm (22700):

IR (KBr) ;1220cm-1 (C-O), 1640cm-1 (C=C), 1790cm-1 (C=O), 2120 cm^{-1}(N_3)

PMR (DMSO); δ 5.88(s, 1H), δ 3.89(q, 2H), δ1.34 (t, 3H); MS; 247 (M+)

4-Azido-3-bromocrotonolactone(VIII)

To an ice cold solution of sodium azide(100mg,0.0016mol)in acetonitrile(10mL)was added 3,4-dibromocrotonolactone(200mg, 0.0008mol)in 2mL acetonitrile and stirred overnight at room temperature. It was diluted with water and extracted with 50mlether. The ether layer was washed with sodium thiosulphate and then with water. The solvent was dried and evaporated to give needle shaped crystals, which was purified by crystallization from hexane to get 50% of the product. M.p 60^{o}C.

UV (EtOH); $\lambda_{max;;}$(ε)268nm (11500),

IR(KBr); 1230cm^{-1} (C-O), 1625 cm^{-1} (C=C), 1750 cm^{-1} (C=O), 2131cm^{-1} (N3),

PMR(DMSO); δ 5.19(s,2H),

MS; 203 (M^+)

4-Amino-3-bromo-5-benzoyloxycrotonolactone(IX)

A mixture of 5-Benzoyloxy-3,4-dibromocrotonolactone(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^{o}C

UV (95%EtOH); $\lambda_{max;}$ (ε)269 nm (16400), 235nm(14900)

IR (KBr) ;1610 cm-1 (C=C), 1730cm-1 (ester C=O), 1775cm-1 (lactone C=O), 3410 and 3225

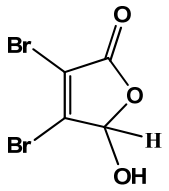
cm^{-1}(N-H)

MS; 229 (M+)

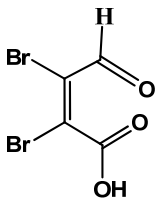
Anal: calculated for $C_{11}H_8BrO_4$: C, 44.31 : H, 2.70: N,4.69. Found: C, 44.32: H,2.96: N4.59.

RESULTS AND DISCUSSION

Mucohalic 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 pencillic acid which showed 225nm in aqueous solution due to pseudo acid form and shifted to 295nm in 0.1N alkali[11]



[1]



[2]

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 ^{0}C, which was characterized as 3,4-dibromo-5-benzoyl-oxycrotonolactone(II) by spectral and analytical methods. Mucobromic acid was reduced with $NaBH_4$ in water at 0^{0}C and gave the product 3,4 dibromocrotonolactone(III). Refluxing mucobromic acid with urea in methanol gave a white solid. m.p 170^{0}C. The compound showed IR bands at 1614, 1700 and 1750 cm^{-1} corresponding to C=C, amide carbonyl and lactone carbonyl respectively along with bands at 3250, 3372, and 3470 corresponding to NH_2 and OH. In mass spectra the molecular ion was apparently absent but peak corresponding to M - $CONH_2$ was observed. Spectral data along with elemental analysis showed the structure as 1-carboxamido-3,4-dibromo-5-hydroxy-3-pyrroline-2-one(V).

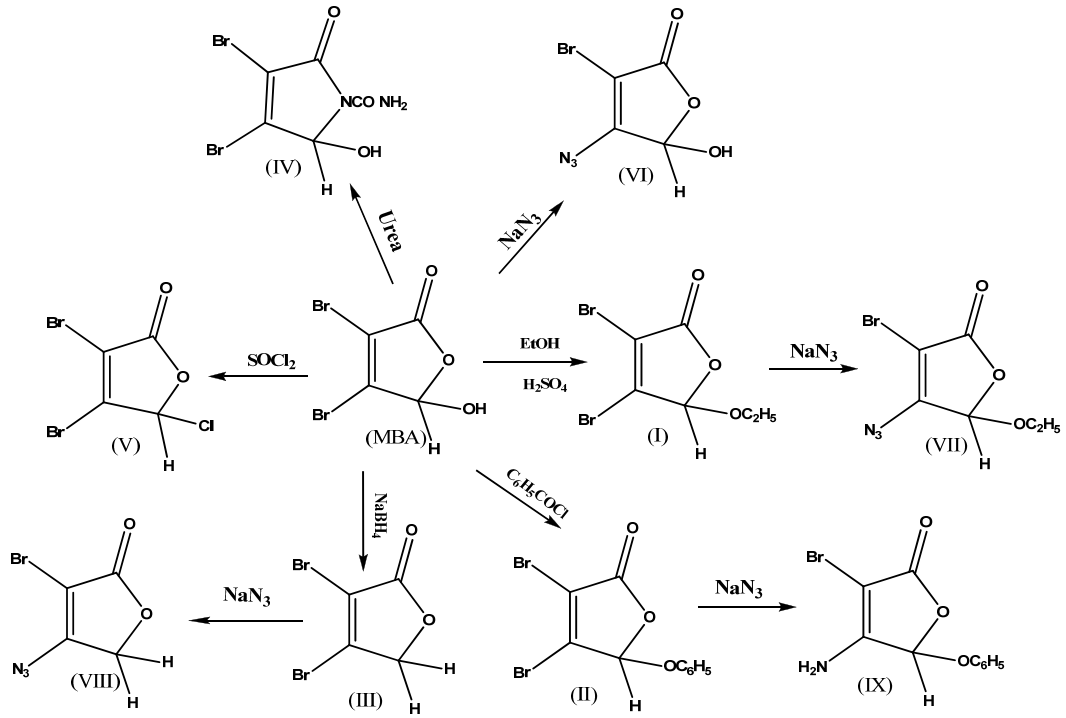
Reaction of mucobromic acid with sodium azide in acetonitrile at room temperature gave solid with melting point 77^{0}C. IR spectra of the compound showed unsaturation at 1625cm^{-1} and lactone carbonyl at 1760cm^{-1} along with strong azide band at 2120cm^{-1} indicating azide substitution. The azide group can react with the acyclic form of mucobromic acid [2] giving an intermediate with azide in the α position of the carbonyl group followed by elimination of bromine atom from the same α carbon. Alternate way is the attack of nucleophile in the pseudo acid form [1] at the β position of the

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 psuedoester could give only the β substituted product, the product was identified as 4-azido-3-bromo-5-ethoxycrotonolactone (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 CD_3CN, which is attributed by the proton in the pseudo form. Thus in acetonitrile, the acid exists in the furanone form and the product was identified as 4-azido-3-bromo-5-hydroxycrotonolactone (VI)

Reaction of lactone with sodium azide gave needle shaped solid of m.p 60 ^{0}C . Mass spectrum showed peaks corresponding to M^+ and M+2 respectively. NMR showed singlet at δ 5.19 corresponding to two hydrogen atoms. Λ max at 268nm confirms azide group on the β carbon to the carbonyl group and the product was 4-azido-3-bromocrotonolactone (VIII).

Reaction of benzoyloxy derivative with sodium azide in acetonitrile gave a dark brown liquid which when extracted with ether gave a yellow solid which showed absorptions characteristic of primary amino group at 3140, 3225 cm^{-1}, and two carbonyl absorptions at 1775 and 1730cm^{-1} corresponding to lactone and ester carbonyls respectively. The product was 4-amino-3-bromo-5-benzoyl-oxycrotonolactone (IX). The azide group may be converted into amine probably through a nitrene intermediate.



Scheme 1

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) [13,14,15] and paper disc diffusion technique [16]. 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^{o}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 bacterial 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 stains.

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

Bacterial strains	Diameter of inhibition zone (mm) for various samples					
	MBA	**I**	**II**	**III**	**IV**	**V**
E.coli	15	17	15	14	9	18
P.vulgaris	10	16	12	12	8	20
P.aeruginosa	13	23	18	15	7	24
S.aureus	9	12	10	9	7	13

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REFERENCES

1. Jones JB, Young JM Carcinogenicity of Lactones III: The Reactions of Unsaturated 4-Lactones with L-Cysteine. Journal of Medicinal Chemistry 1968; 11: 1176.
2. Dickens F, Jones HEH Further studies on the carcinogenic action of certain lactones and related substances in the rat and mouse. Brit J Cancer1965; 19: 392.
3. Harry H Wasserman, Frank M Precopio, Tien-Chuan Liu, Studies on the Mucohalic Acids II: The Synthesis of Fused α-Lactam-thiazolidines Related to Penicillin. J Am Chem Soc 1952; *74* (16): 4093.
4. Allen CFH, Spangler FW Organic Synthesis, Vol 3. John Wiley and Sons,Inc: New York; 1967.
5. Taylor GA Organic Synthesis, Vol 4. John Wiley and Sons, Inc: New York; 1964.
6. Simonis H, Safmony A New Methods in the Chemistry Pyridazones I-phenyl derivative by treating mucobromic acid with hydrazine hydrate or phenyl hydrazine respectively. Ber dtsch Chem Ges 1905; 38: 2588.
7. Mowry D T Mucochloric Acid I: Reactions of the Pseudo Acid Group. J Am Chem Soc 1950; *72*: 2535.
8. Fabio Bellina, Renzo Rossi Mucochloric and Mucobromic Acids: Inexpensive Highly Functionalised Starting Materials for the Selective Synthesis of Variously Substituted 2(5H)-Furanone Derivatives, Sulfur- or Nitrogen-Containing Heterocycles and Stereo defined Acyclic Unsaturated Dihalogenated Compounds. Current Organic Chemistry 2004; 8, (15): 121089.
9. Kurbangalieva AR, Devyatova NF Bogdanov AV, Berdnikov EA, Mannafov TG, Krivolapov DB, Litvinov IA, Chmutova GA Synthesis of Novel Arylthio Derivatives of Mucochloric Acid: Phosphorus, Sulfur, and Silicon and the Related Elements 2007; 182, 3: 607**.**
10. John T Braunholtz, Keith B Mallion ,Frederick G Mann The structure and properties of certain polycyclic indole- and quinolino-derivatives. Part XIV: Derivatives of 2, 3-dihydro-1-oxo-1*H*-pyrrolizine. J Chem Soc 1962: 4346.
11. Shaw E Patulin and Penicillic Acid. J Amer Chem Soc 1946; 68: 2510.
12. Harry H Wasserman, Frank M Precopio Studies on the Mucohalic Acids. IV: Replacement of Halogen in the Pseudo Ester Series. J Am Chem Soc. 1954; 76 (5):1243.
13. Villanova PA. National Committee for clinical and Laboratory Standards, Methods for dilution antimicrobial susceptibility tests for bacteria. NCCLS Document M7-A4; 1997.
14. Isenberg DH, Essential Procedure for Clinical Microbiology. American Society for Microbiology; Washington: 1998.
15. Zgoda JR, Porter JR A Convenient Microdilution Method for Screening Natural Products Against Bacteria and Fungi. Pharm. Biol. 2001; 39: 221.
16. Wayne PA NCCLS: DISC-Diffusion Eleventh International Supplement, NCCLS Document. M100-S11, 2001.