Srinivas Kantham /J. Pharm. Sci. & Res. Vol.3(6), 2011,1284-1287

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Antimicrobial Activity of Chemoenzymatically Prepared Ribofuranose Derived Cationic Surfactants

Srinivas Kantham

Department of Pharmaceutical Chemistry, S.R.L Institute of Pharmaceutical Sciences, Affiliated to Kakatiya University, Kummarigudem Road, Madikonda, Kazipet, Warangal, A.P-506003 - India.

Abstract:

Aims: In order to increase biodegradation ability, hydrophilicity, compatibility of the surfactant, with other ingredients, and to enhance their antimicrobial activity, we prepared 2-[3-(N,N,N-trimethylamino)propoxy]- 5-O-alkanoyl-D-ribofuranoside derivative by simple Chemoenzymatic methods.

Methods: Surface active properties with different fatty acid side chain were estimated using Wilhelmy Plate method by Sigma 70 tensiometer at 25^0C; The antimicrobial activity of all the newly synthesized compounds were determined by well plate method in nutrient agar (Hi-Media) was used for antibacterial activity.

Results: In this study we found that tetradecanoyl and hexadecanoyl esters highest ability in lowering surface tension as well as lowest critical micelle concentration values. Interestingly the newly prepared surfactants showed good antimicrobial activity. The most potent compounds dodecanoyl, decanoyl esters are able to inhibit both (Two Gram-Positive Bacteria: *Bacillus subtilis* and *Staphylococcus* aureus and two Gram-Negative Bacteria: *Escherichia coli* and *Proteus vulgaris)* bacterial strain and fungal test strains (*Aspergillus niger*, *Candida albicans*, *Trichosporon beigelli* and *Aspergillus flavus*).

Conclusion: The antibacterial and antifungal activities of synthesized compounds were comparable with Ciprofloxacin and Griseofulvin respectively.

Keywords*:* Antimicrobial activity, Cationic surfactants, D-Ribofuranose

INTRODUCTION

A number of carbohydrates, including sucrose, have been used for the chemical synthesis of surfactants [1]. An important class of cationic surfactants is the quaternary ammonium salt, which is used within specialized areas such as fabric softeners, hair conditioners, and antimicrobial agents. Most quaternary ammonium cationic surfactants are limited in their use due to their poor compatibility and biodegradation [2].

In this project we planned to develop new Furanose based surfactants chemo-enzymatically to increase biodegradation ability, hydrophilicity, compatibility of the surfactant, with other ingredients, and to enhance their antimicrobial activity, there is a tremendous need to develop a new carbohydrate-based surfactants utilizing commercially available and cost-effective carbohydrates and heterocycles as raw materials, with environmentally benign chemo-enzymatic reaction conditions [3].

In this contest, the incorporation of a carbohydrate (Ribofuranose) moiety appears very attractive because the hydrophilic groups are very concentrated and are biodegradable. New types of carbohydrate based surfactants are synthesized chemo-enzymatically, as outlined in Scheme 1.

EXPERIMENTAL PROCEDURES:

Melting points were determined in open capillary tubes, using Toshniwal melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Brucker spectrospin 300 MHz spectrophotometer using TMS as an internal standard. Purity was checked by TLC using TLC aluminum sheets silica gel 60, supplied by Merk, Mumbai, India.

Synthesis of 2-(3-chloropropoxy)-D- ribofuranoside (2): The Ribofuranose (1) (15g 99.91mmol) and acidic ion exchange resin were suspended by stirring in 3-chloro-1-propanol (100mL, 1.19mol) at 60 ^{0}C. Completion of the reaction was monitored by TLC. Then the reaction mixture was purified by silica gel column chromatography using diethyl ether/ethyl acetate/methanol to afford colorless viscous liquid in 82.15% yield (18.6g).

^{1}H NMR (300 MHz, D_2O): 1.70-1.75 (m, 2H), 3.20-3.50 (m, 2H), 3.30-3.32 (m, 4H), 3.60-3.80 (m, 2H), 4.00-4.10 (m, 2H), 4.80 (t, 3H), 4.47 (0.3H, d,J=7.4Hz, H-1β), 5.22 (0.7H, d, J=3.6Hz, H-1α).

Synthesis of 2-(3-chloropropoxy)-5-O-alkanoyl-D-ribofuranoside (3a-e): *General Procedure*: Compound 2 (2g, 8.82mmol) was dissolved in melted fatty acid (e.g., Decanoic acid, 1.52g, 8.82mmol) at 80^0C. Immobilized C. Antarctica B-lipase was added (5g), and the mixture was stirred for 26 hr under reduced pressure. The reaction mixture was diluted with acetone (100mL), filtered and concentrated. The product was purified by silica gel column chromatography using diethyl ether/ethyl acetate/methanol and it was confirmed by ^{1}H NMR. All the compounds (3a-e) are in colorless viscous liquid in nature and are afforded in different yields (3a: 83.25%; 3b: 82.20%; 3c: 85.10%; 3d: 80.45%; 3e: 86.20%)

Synthesis of 5-(3-chloropropoxy)-3,4-dihydroxy-tetrahydrofuran-2-yl)methyl decanoate (3a): ^{1}H NMR (300 MHz, D_2O): 0.82 (t, 3H), 1.30-1.32 (m,

12H), 1.69-1.74 (m, 4H), 2.12-2.25(m, 2H), 3.30-3.34 (m, 4H), 3.60-3.80 (m, 2H), 4.30-4.35 (m, 3H), 4.80 (s, 2H), 5.10 (0.3H, d,J=7.4Hz, H-1β), 5.13 (0.7H, d, J=3.6Hz, H-1α).

Synthesis of 5-(3-chloropropoxy)-3,4-dihydroxy-tetrahydrofuran-2-yl)methyl dodecanoate (3b): ^{1}H NMR (300 MHz, D_2O): 0.86 (t, 3H), 1.25-1.30 (m, 16H), 1.68-1.74 (m, 4H), 2.10-2.2 m, 2H), 3.30-3.35 (m, 4H), 3.60-3.80 (m, 2H), 4.30-4.35 (m, 3H), 4.80 (s, 2H), 5.10 (0.3H, d,J=7.4Hz, H-1β), 5.13 (0.7H, d, J=3.6Hz, H-1α).

Synthesis of 5-(3-chloropropoxy)-3,4-dihydroxy-tetrahydrofuran-2-yl)methyl tetradecanoate (3c): ^{1}H NMR (300 MHz, D_2O): 0.86 (t, 3H), 1.26-1.33 (m, 20H), 1.66-1.73 (m, 4H), 2.12-2.25(m, 2H), 3.30-3.35 (m, 4H), 3.60-3.70 (m, 2H), 4.30-4.35 (m, 3H), 4.80 (s, 2H), 5.10 (0.3H, d,J=7.4Hz, H-1β), 5.13 (0.7H, d, J=3.6Hz, H-1α).

Synthesis of 5-(3-chloropropoxy)-3,4-dihydroxy-tetrahydrofuran-2-yl)methyl hexadecanoate (3d): ^{1}H NMR (300 MHz, D_2O): 0.85 (t, 3H), 1.30-1.35 (m, 24H), 1.70-1.75 (m, 4H), 2.12-2.25(m, 2H), 3.30-3.34 (m, 4H), 3.60-3.80 (m, 2H), 4.30-4.34 (m, 3H), 4.80 (s, 2H), 5.10 (0.3H, d,J=7.4Hz, H-1β), 5.13 (0.7H, d, J=3.6Hz, H-1α).

Synthesis of 5-(3-chloropropoxy)-3,4-dihydroxy-tetrahydrofuran-2-yl)methyl octadecanoate (3e): ^{1}H NMR (300 MHz, D_2O): 0.86 (t, 3H), 1.30-1.35 (m, 28H), 1.70-1.80 (m, 4H), 2.12-2.25(m, 2H), 3.30-3.34 (m, 4H), 3.60-3.80 (m, 2H), 4.30-4.35 (m, 3H), 4.80 (s, 2H), 5.10 (0.3H, d,J=7.4Hz, H-1β), 5.13 (0.7H, d, J=3.6Hz, H-1α).

Synthesis of 2-[3-(N,N,N-trimethylamino)propoxy]-5-O-alkanoyl-D-ribofuranoside iodine salt (4a-e): *General Procedure*: Compound 3 (5g, 13.12mmol) and sodium iodide (1g, 6.67mmol) was dissolved in 2-butanone (50mL) and the solution was boiled for 28 hr. The precipitated sodium chloride was removed by filtration. Trimethylamine (5mL, 56.6mmol) was cooled to 0^0C and then added to above solution with constant stirring and the completion of the reaction was monitored by TLC. The solvent was removed under reduced pressure to afford the colorless viscous product in 85% yield [4].

5-O-decanoyl-2-[3-(N,N,N-trimethyl amino) propoxy] -D-ribofuranoside iodine salt (4a): ^{1}H NMR (300 MHz, D_2O): 0.85 (t, 3H), 1.22-1.29 (m, 12H), 1.58-1.65 (m, 2H), 1.70-1.75 (m, 2H), 2.12-2.25(m, 2H), 3.10 (s, 9H), 3.3 (m, 4H), 3.60-3.80 (m, 2H), 4.20-4.30 (m, 3H), 4.70 (s, 2H), 4.91 (0.3H, d,J=7.4Hz, H-1β), 5.13 (0.7H, d, J=3.6Hz, H-1α).

5-O-dodecanoyl-2-[3-(N,N,N-trimethylamino)propoxy]-D-ribofuranoside salt (4b): ^{1}H NMR (300 MHz, D_2O): 0.85 (t, 3H), 1.20-1.30 (m, 16H), 1.58-1.65 (m, 2H), 1.64-1.70 (m, 2H), 2.10-2.25(m, 2H), 2.90 (s, 9H), 3.30-3.15 (m, 4H), 3.60-3.80 (m, 2H), 4.20-4.30 (m, 3H), 4.70 (s, 2H), 4.85 (0.3H, d,J=7.4Hz, H-1β), 5.10 (0.7H, d, J=3.6Hz, H-1α).

5-O-tetradecanoyl-2-[3-(N,N,N-trimethylamino)propoxy]-D-ribofuranoside salt (4c): ^{1}H NMR (300 MHz, D_2O): 0.85 (t, 3H), 1.22-1.29 (m, 18H), 1.58-1.65 (m, 2H), 1.70-1.75 (m, 2H), 2.12-2.25(m, 2H), 2.90 (s, 9H), 3.40-3.45 (m, 4H), 3.60-3.80 (m, 2H), 4.20-4.30 (m, 3H), 4.70 (s, 2H), 4.91 (0.3H, d,J=7.4Hz, H-1β), 5.13 (0.7H, d, J=3.6Hz, H-1α).

5-O-hexadecanoyl-2-[3-(N,N,N-trimethylamino)propoxy]-D-ribofuranoside iodine salt (4d): ^{1}H NMR (300 MHz, D_2O): 0.85 (t, 3H), 1.22-1.29 (m, 20H), 1.58-1.65 (m, 2H), 1.70-1.75 (m, 2H), 2.12-2.25(m, 2H), 3.10 (s, 9H), 3.33-3.38 (m, 4H), 3.60-3.80 (m, 2H), 4.20-4.30 (m, 3H), 4.70 (s, 2H), 4.91 (0.3H, d,J=7.4Hz, H-1β), 5.13 (0.7H, d, J=3.6Hz, H-1α).

5-O-octadecanoyl-2-[3-(N,N,N-trimethylamino)propoxy]-D-ribofuranoside iodine salt (4e): ^{1}H NMR (300 MHz, D_2O): 0.85 (t, 3H), 1.22-1.29 (m, 22H), 1.58-1.65 (m, 2H), 1.70-1.75 (m, 2H), 2.12-2.25(m, 2H), 3.10 (s, 9H), 3.36-3.40 (m, 4H), 3.60-3.80 (m, 2H), 4.20-4.30 (m, 3H), 4.70 (s, 2H), 4.91 (0.3H, d,J=7.4Hz, H-1β), 5.13 (0.7H, d, J=3.6Hz, H-1α).

SURFACE ACTIVE PROPERTIES:

Surface tension of surfactant solutions was determined, using Wilhelmy Plate method by Sigma 70 tensiometer at 25^0C. The critical micelle concentration (CMC) was determined from the break point of each surface tension vs. concentration. Foaming properties were measured using Ross- Miles Test at ambient temperature using 1g/L solution of the test solution in distilled water [5].

ANTIMICROBIAL ACTIVITY:

The antimicrobial activity of all the newly synthesized compounds were determined by well plate method in nutrient agar (Hi-Media) was used for antibacterial activity. The antibacterial activity of the test compounds was assayed against *Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 96 (Gram Positive) and *Escherichia coli* MTCC 722, *and Proteus vulgaris* MTCC 109 (Gram Negative) by CUP-Plate method [6].

The compounds were tested at a concentration of a 100µg/ml were prepared in dimethylformamide (DMF). The Petri dishes used for antibacterial screening were incubated at 37 ± 1º for 24 h; the diameters of zone of inhibition (mm) surrounding each of the wells were recorded. The results were compared with Ciprofloxacin of a 100µg/ml concentration. The antifungal activity of test compounds was determined against four fungal strains; *Aspergillus niger* ATCC6275, *Candida albicans* ATCC2091, *Trichosporon beigelli* NCIM3404 and *Aspergillus flavus* NCIM538. All the bacterial and fungal strains were obtained from Kakatiya University, Warangal, India.

RESULTS AND DISCUSSION:

The synthesis of 5-O-estersm of-2-[3-(N,N,N-trimethylamino)propoxy]-D-ribofuranoside (4a-e) are outlined in the Scheme 1. Accordingly, treatment of Ribofuranose **1** with chlorohydrins to give respective ethers **2**, selective esterification of primary alcohol in the glycosides can be achieved by a very simple lipase-catalyzed (from *C. Antarctica*) process using the molten fatty acid as solvent (Dodecanoic, Tetradecanoic, Hexadecanoic, Octadecanoic acids). The resulting products **3** can be treated with sodium iodide fallowed by trimethylamine to afford new cationic surfactants **4** (quaternary ammonium salt) in good yield.

The title compounds 4a-e found to have excellent surface active properties, shown in Table 1. Compounds 4a, 4b, and 4c (containing decanoyl, dodecanoyl, and tetradecanoyl esters respectively), exhibiting highest ability to lower surface tension as well as having lowest CMC values. The compound 4a showed equivalent foaming properties when compared with SDS, which were evaluated with standard Ross- Miles test.

Scheme 1: Preparation of Ribofuranose Based Surfactants.

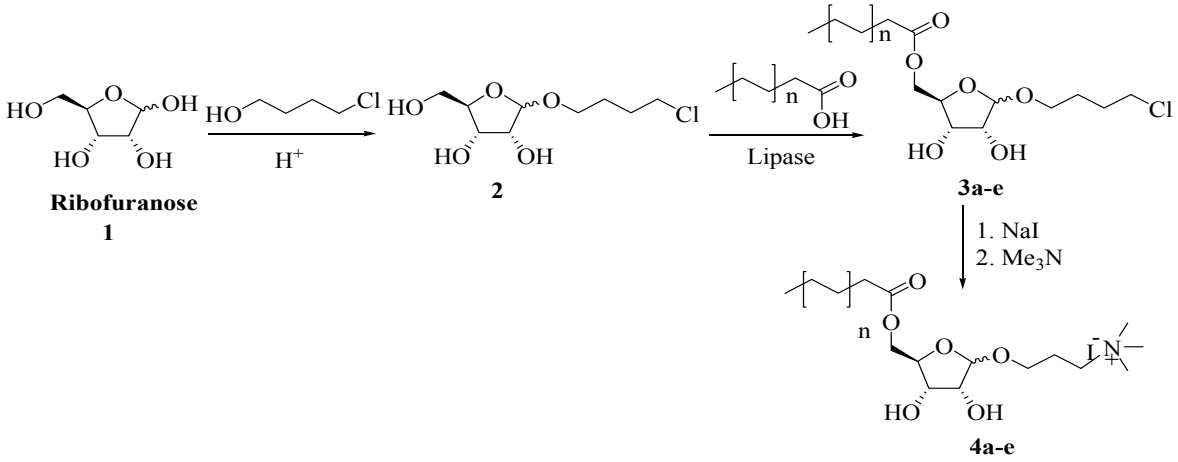

Table 1: S urface Active properties of 5-O-alkanoyl-2-[3-(N,N,N-trimethylamino)propoxy]-D- ribofuranoside iodine salts (4a-e)

Compound	n	5-O-Acyl side chain	CMC (10^{-4} mol/L)	γ_{min} (mN/m)	Temp. (^{0}C)
4a	8	Decanoyl	0.20	15.60	25
4b	10	Dodecanoyl	0.50	23.60	26
4c	12	Tetradecanoyl	0.67	25.40	27
4d	14	Hexadecanoyl	1.90	37.40	27
4e	16	Octadecanoyl	1.14	40.20	27

Table 2: Data on antimicrobial activity of 5-O-alkanoyl-2-[3-(N,N,N-trimethylamino)propoxy]-D-ribofuranoside iodine salts (4a-e)

Compound (100µg/mL)	Antibacterial Activity (Zone of inhibition in mm)				Antifungal Activity (Zone of inhibition in mm)			
	B. subtilis	***S. aureus***	***E. coli***	***P. vulgaris***	***A. niger***	***C. albicans***	***T. beigelli***	***A. flavus***
4a	22	20	20	18	19	19	26	15
4b	22	20	18	20	22	21	28	16
4c	19	18	17	18	20	19	19	16
4d	13	12	17	16	18	15	21	14
4e	20	22	20	18	20	20	23	20
Control	-	-	-	-	-	-	-	-
Ciprofloxacin	22	20	18	18	-	-	-	-
Griseofulvin	-	-	-	-	21	22	28	16

The new surfactants were able to inhibit both bacterial strain (Two Gram-Positive Bacteria: *Bacillus subtilis* and *Staphylococcus aureus* and two Gram-Negative Bacteria: *Escherichia coli* and *Proteus vulgaris*) and fungal test strains (*Aspergillus niger*, *Candida albicans*, *Trichosporon beigelli* and *Aspergillus flavus*). The most potent compounds 4b, 4a, 4e (decanoyl, dodecanoyl, and octadecanoyl esters) antibacterial and antifungal activities of synthesized compounds were comparable with Ciprofloxacin and Griseofulvin respectively. Antimicrobial activity of compounds 4a-e was showed in Table 2.

CONCLUSION

A vast variety of new carbohydrate-based surfactants can be easily achieved utilizing commercially available and cost-effective carbohydrates and heterocycles as raw materials, with environmentally benign chemo-enzymatic protocols with enhanced biodegradation ability, hydrophilicity, compatibility of the surfactant, with other ingredients, and to enhance their antimicrobial activity. Furthermore, as the carbohydrate ester-based products are expected to be readily biodegradable, these properties should encourage further optimization and studies toward commercialization.

ACKNOWLEDGMENTS

The authors are thankful to the Director and Principal, S.R.L Institute of Pharmaceutical Sciences, Madikonda, Kazipet, Warangal, India, Kakatiya University for providing laboratory facilities and financial support.

REFERENCES

[1] Dhananjoy, M.; Zhanel, G.G.; Schweizer, F., *Carbohydr. Res*. 2011, 346(5), 588-594.

[2] Thebault, P.; Givenchy, E.T.; Géribaldi, S.; Richard, L.; Vandenberghe, y.; Guittard, F., *J. Fluorine Chem*. 2010, 131(5), 592-596.

[3] Nichifor, M.; Stanciu, M.C.; Simionescu, B.C., *Carbohydr. Polym.* 2010, 82(3), 965-975.

[4] Shigetomi, K.; Shoji, K.; Mitsuhashi, S.; Ubukata, M., *Phytochem. Lett*.2010, 71(3), 312-324.

[5] Negm, N.A.; Zaki, M.F.; Salem, M.A.I., *Colloids Surf. B*. 2010, 77(1), 96-103.

[6] Zander, J.; Besier, S.; Faetke, S.; Saum, S.M.; Müller, V.; Wichelhaus, T. A., *Int. J. Antimicrob. Agents*. 2010, 36(6), 562-565.