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SYNTHESIS AND ANTIBACTERIAL ACTIVITIES OF SOME URIDINE DERIVATIVES

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ABSTRACT

Regioselective cinnamoylation of uridine using the dibutyltin oxide method furnished the corresponding 3'-*O*-cinnamoate in reasonable yield. Whereas, direct method afforded the 5'-*O*-cinnamoate, though in somewhat lower yield. 5'-*O*-(3-Bromobenzoyl)-2',3'-di-*O*-acetyluridine and 5'-*O*-(4-chlorobenzoyl)-2',3'-di-*O*-acetyluridine were also prepared. Antibacterial activities of these and a number of previously synthesized derivatives of uridine against three human pathogens viz. *Bacillus subtilis* BTCC 17, *Salmonella typhi* AE 14612 and *Eschericia coli* ATCC 25922 have been studied *in vitro*. These uridine derivatives display mild antibacterial activities against all the test bacteria in comparison to uridine.

ইউরিডিনকে ডাইবিউটাইলটিন অক্সাইড পদ্ধতিতে সিনাময়লেশন করলে পরিমিত পরিমানে ৩′-*O*-সিনাময়েট পাওয়া যায়। অথচ, সরাসরি পদ্ধতিতে ৫′-*O*- সিনাময়েট পাওয়া যায়, যদিও পরিমাণে কিছু কম। ৫′-*O*–[৩′-ব্রোমোবেনযোয়েল)-২′, ৩′-ডাই-*O*- অ্যাসিটাইলইউরিডিন এবং ৫′, *O*- (৪′- ক্লোরোবেনযোয়েল) – ২′, ৩′- ডাই *O*–অ্যাসিটাইল ইউরিডিনও তৈরি করা হয়েছে। মানব রোগের তিনটি জীবানু; যথাঃ *Bacillus subtilis* BTCC ১৮, *Salmonella typhi AE* ১৪৬১২ and *Eschericia coli* ATCC ২৫৯২২ এর বিরুদ্ধে এইসব এবং পূর্বে সংশ্লেষনকৃত কতগুলি ইউরিডিন জাতকের *in vitro* পদ্ধতিতে ব্যাকটিরিয়া বিরোধী সক্রিয়তা নিরূপন করা হয়েছে। ইউরিডিনের এই জাতকসমূহ পরীক্ষণীয় ব্যাকটেরিয়া সমূহের বিরুদ্ধে ইউরিডিন অপেক্ষা মৃদু সক্রিয়তা প্রদর্শন করে।

INTRODUCTION

It is well established that most nucleoside analogues become biologically active [1]. For this reason, there has been increasing interest in the synthesis of various uncleoside derivatives, which might act as antiviral agent [2] but with greatly reduced toxicity. Various methods for acylation of carbohydrates and nucleosides have so far been developed and employed successfully [3-7]. Of these, the dibutyltin oxide was found to be the most encouraging versatile reagent. In the present research project, we synthesized various acylated uridine derivatifes by dibutyltin oxide method and direct methods and evaluated their *in vitro* antibacterial activities.

EXPERIMENTAL

Physical measurements

Melting points were determined on an electrothermal melting point apparatus and are uncorrected. All reagents used were commercially available (Aldrich) and were used as received, unless otherwise specified. Evaporations were performed under diminished pressure on a Buchi rotary evaporator. IR spectra were recorded on a Perkin–Elmer IR–883 spectrophotometer using the KBr technique. ^{1}H–NMR spectra (200 MHz) were recorded for solutions in deuteriochloroform (internal Me_4Si) with a Bruker spectrometer. Anlytical thin layer chromatography (t. l. c.) was performed on Kieselgel GF_{254}. Visualization of spots was accomplished by spraying the plates with 1% H_2SO_4 followed by heating plates at 150–200°C until colouration took place. Column chromatography was performed with silica gel G_{60}. Solvent system employed for t. l. c. analyses was methanol – chloroform in different proportions.

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3'–O–Cinnamoyluridine, 3

Dibutyltin oxide method:

A solution of uridine (**1**) (200 mg, 0.82 mmol) in anhydrous methanol (10 ml.) was treated with dibutyltin oxide (224 mg, 1.1 molar eq) and the suspension was heated under gentle reflux under nitrogen for 4 hrs. and the solvent was removed under reduced pressure. The resulting white tin complex was then dissolved in dry 1,4-dioxane (12 ml) and cinnamoyl chloride (150 mg, 1.1 molar eq) was added to it. The mixture was stirred at room temperature for 18 hrs. The progress of the reaction was monitored by t. l. c. (methanol – chloroform, 1:5, R_f=0.51) showed formation of a single product. Removal of solvent and purification by column chromatograhpy (methanol - chloroform, 1:5 as eluant) gave the title compound (**3**) (272 mg, 89%) as needles, mp. 136-137°C (hexane).

Anal. calcd. for $C_{18}H_{18}O_7N_2$: C, 57.79; H, 4.85.
Found: C, 57.61; H, 4.82%.
IR (KBr) : 1630 (–CH=CH–), 1680, 1720 (–CO), 3510 cm^{-1} (–OH).
^{1}H–NMR : δ_H 8.05 (1H, d, J= 7.8 Hz, H–6), 7.80 (1H, d, J=16.0 Hz, PhC*H*=CHCO–), 7.65 (2H, m, Ar–H), 7.42 (3H, m, Ar–H), 6.68 (1H, d, J= 16.0 Hz, PhCH=C*H*CO) 6.02 (1H, d, J=5.8 Hz, H–1'), 5.72 (1H, d, J=7.8 Hz, H–5), 5.32 (1H, m, H–3'), 4.50 (1H, m, H–2'), 4.25 (1H, m, H–4'), 3.82 (2H, m, H–5').

5'–O–Cinnamoyluridine, 4

Direct method:

A cooled (0°C) and stirred solution of uridine (**1**) (300 mg, 1.23 mmol) in dry pyridine (6 ml) was treated with cinnamoyl chloride (225 mg. 1.1 molar eq). The solution was stirred at 0°C for 6 hrs. and then kept in a refriegerator overnight when t. l. c. (methanol – chloroform, 1:4, R_f=0.49) indicated full conversion of the starting material into a single product. A few pieces of ice was added to the flask with constant stirring and the contents extracted with chloroform (3 x 15 ml). The combined chloroform extract was washed successively with dil. HCI, saturated $NaHCO_3$ and water. The organic layer was dried ($MgSO_4$) and concentrated. Percolation of the residue through a silica gel column, with methanol – chloroform, 1:8 as eluant provided compound **4** (313 mg, 68%) as needles, mp. 108–109°C (chloroform – hexane).

Anal. calcd. for $C_{18}H_{18}O_7N_2$: C, 57.79; H, 4.85.
Found: C, 57.88; H, 4.96%.
IR (KBr) : 1632 (–CH=CH–), 1675, 1710 (–CO), 3510 cm^{-1} (–OH).
^{1}H–NMR : δ_H 8.00 (1H, d, J= 8.0 Hz, H–6), 7.82 (1H, d, J=16.0 Hz, PhC*H*=CHCO–), 7.71 (2H, m, Ar–H), 7.51 (3H, m, Ar–H), 6.71 (1H, d, J=16.0 Hz, PhCH=C*H*CO–), 5.98 (1H, d, J=5.5 Hz, H–1'), 5.75, (1H, d, J=8.0 Hz, H–5), 5.68 (2H, m, H–5'), 4.55 (1H, m, H–2'), 4.48 (1H, m, H–3'), 4.31 (1H, m, H–4').

5'–O–(3–Bromobenzoyl)–2', 3'–di–O–acetyluridine, 8

To a cooled and stirred solution of the diol **7** [8] (70 mg, 0.16 mmol) in dry pyridine (3 ml) was added acetic anhydride (0.08 ml, 5 molar eq) and stirring was continued at room temperature for 8 hrs. A few pieces of ice was added to the flask with constant stirring and the contents extracted with chloroform. The chloroform extract was washed successively with dil. HCI, saturated $NaHCO_3$ and water. The organic layer was dried ($MgSO_4$) and concentrated. Percolation of the residue through a silica gel column, with methanol – chloroform (1:12) as eluant (R_f=0.52), provided the diacetate **8** (72 mg, 85%) as needles, mp.103–104°C.

IR (KBr): 1675, 1712, 1722 cm^{-1} (–CO).
^{1}H–NMR : δ_H H 8.00 (1H, s, Ar–H), 7.98 (1H, d, J=7.7 Hz, Ar–H), 7.93 (1H, d, J=8.0 Hz, H–6), 7.85 (1H, d, J=7.8 Hz, Ar–H), 7.51 (1H, t, J=5.5 Hz, Ar–H), 5.80 (1H, d, J=5.6 Hz, H-1'), 5.73 (2H, m, H–5'), 5.66 (1H, d, J=8.0 Hz, H–5), 5.58 (1H, d, J=5.6 Hz, H–2'), 5.52 (1H, m, H–3'), 4.55 (1H, m, H–4'), 2.08, 1.98 (2 x $3H_2$ x s, 2 x MeCO–).

5'–O–(–4–Chlorobenzoyl)–2',3'–di–O–acetyluridine, ***12***

Acetylation of the diol **11** [8] (70 mg, 0.18 mmol) with acetic anhydride (0.081 ml, 5 molar eq) in pyridine, using the procedure as described erlier, followed by chromatographic purification (methanol – chloroform, 1: 10) (R_f=0.51) furnished the diacetate **11** in 88% yield as needles, mp. 128–129°C.

IR (KBr) :1667, 1710, 1725 cm^{-1}(–CO).
^{1}H–NMR : δ_H 7.90 (1H, d, J= 8.0 Hz, H–6), 7.50 (2H, d, J= 7.8 Hz, Ar–H), 7.37 (2H, d, J= 7.8 Hz, Ar–H), 5.95 (1H, d, J= 5.6 Hz, H–1'), 5.72 (2H, m, H–5'), 5.68 (1H, d, J = 8.0 Hz, H–5), 5.63 (1H, m, H–2'), 5.49 (1H, m, H–3'), 4.48 (1H, m, H–4'), 2.10, 2.05 (2 x 3H, 2 x s, 2 x MeCO).

Antibacterial screening experiments :

The antibacterial activities of uridine **(1)** and some of its acylated derivatives **(3–12)**, as shown in Fig. –1, were determined *in vitro* against three pathogenic microorganisms viz. *Bacillus subtilis* BTCC 17 (Gram- positive), *Salmonella typhi* AE 14612 (Gram-negative) and *Escherichia coli* ATCC 25922 (Gram–negative). For the detection of antibacterial activities, the disc (8 mm) diffusion method described by Bauer *et al.* [9] was followed. Nutrient Agar was used as a basal medium and dimethyl formamide (DMF) was used as a solvent to prepare desired solution (1.0% & 0.5%) of the compounds. The plates were incubated at 37°C for 48 hrs. The antibacterial activities were expressed by measuring diameter zone of inhibition in mm. Proper control was mantained with DMF, Each experiment was carried out three times.

RESULTS AND DISCUSSION

Wagner *et al.* [10] prepared some acylated products of uridine **(1)**, cytidine, adenosine and inosine. They used some acylating agent such as tosyl, benzoyl and acetyl and followed the dibutyltin oxide method. In the present work, we reinvestigated the regiospecificity of uridine **(1)** by dibutyltin oxide method as well as direct method using cinnamoyl chloride, a rarely used acylating agent.

Reaction of uridine **(1)** with dibutyltin oxide in anhydrous methanol under reflux condition (~4 hrs.), followed by removal of solvent furnished the 2', 3'–*O*–dibutylstannylene derivative **(2)** as a white solid. The formation of the stannylene complex **(2)** may be explained by considering that a five membered stannylene ring is formed between the *cis*-glycol system of 2'–OH and 3'–OH and is the most stable [4]. The observation was in conformity with that proposed by Wagner *et al.* [10].

The stannylene derivative **2** was then reacted with cinnamoyl chloride in dry dioxane at room temperature overnight and then chromatographic purification yielded the cinnamoate **3** in 89% yield as needles, mp. 136-137°C. The IR spectrum of this compound showed absorption bands at 1630 (–CH=CH–), 1680, 1720 (–CO) and 3510 cm^{-1} (–OH). The ^{1}H–NMR spectrum contained the following characteristic peaks : δ 7.80 (1H, d, J=16.0 Hz, PhC*H*= CHCO–) and d 6.68 (1H, d, J=16.0 Hz, PhCH=C*H*CO), thereby confirming the presence of one cinnamoyl group in the molecule. The low field resonance of H–3' to d 5.32 was indicative of the attachment of the

cinnamoyl group at position 3'. The rest of the ^{1}H– N M R spectrum was in conformity with the structure accorded to it. The formation of compound **3** may be explained by assuming that cinnamoyl chloride attacks the most reactive and less sterically hindered 3' position, thereby forming the 3'-*O*-cinnamoate as the sole product.

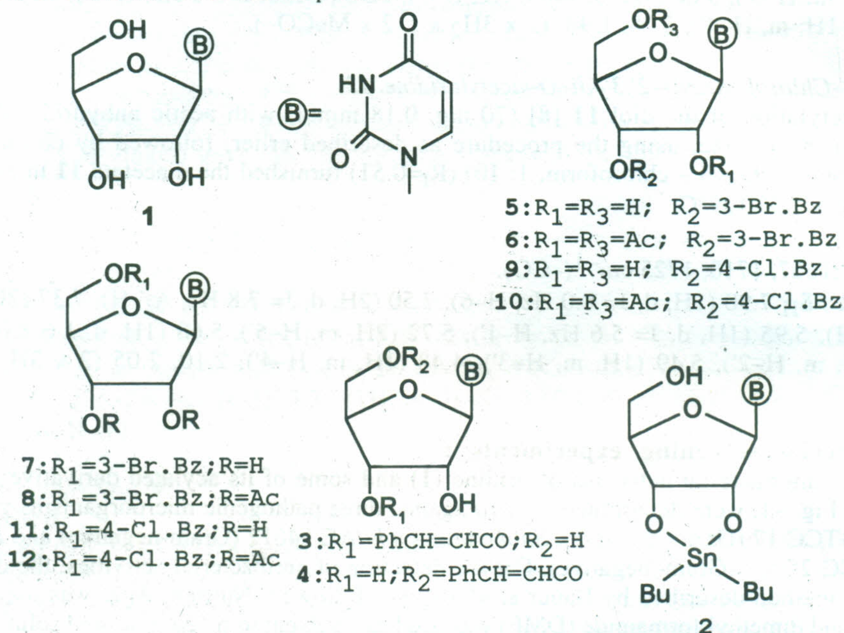

Fig.-1 : Structure of compounds 1-12.

We then reacted uridine (**1**) with cinnamoyl chloride in pyridine at freezing temperature. After usual work-up and chromatographic purification, compound **4** was obtained in 68% yield as needles, mp. 108-109°C. The IR spectrum of compound **4** showed olefinic, carbonyl and hydroxyl stretching bands at 1632, 1675 and 1710 and 3510 cm^{-1}, respectively. The ^{1}H–NMR spectrum showed two characteristic doublets at δ 7.82 (1H, d, J=16.0 Hz, PhC*H*=CHCO–) and δ 6.71 (1H, d, J=16.0 Hz, PhCH=C*H*CO–) and two multiplets at δ 7.71 (2H) and δ 7.51 (3H) indicating the presence of one cinnamoyl group in the molecule. The downfield shift of C–5' protons to δ 5.68 from their usual values [8] indicated the introduction of the substituent at C–5'.

Uridine (**1**), on treatment with 3–bromobenzoyl chloride in pyridine (direct mehtod), yielded compound **7** in 51% yield [8]. Compound **7** was then acetylated with acetic anhydride in pyridine to give the diacetate **8.** The ^{1}H–NMR spectrum of compound **8** showed two three-proton singlets at δ 2.08 and δ 1.98 which were due to the methyl protons of the acetyloxy groups. The deshielding of H–2' and H–3' from their usual values, indicated that the acetyl groups were attached at positions 2' and 3'. Complete analysis of the spectrum was in agreement with the structure accorded to it.

Direct 4-chlorobenzoylation of uridine (**1**) afforded compound **11** in 54% yield [8]. This compound (**11**), on acetylation, furnished the diacetate **12** in 88% yield as needles mp. 128–129°C. In its ^{1}H–NMR spectrum two three-proton singlets at δ 2.10 and δ 2.05 were due to the methyl protons of two acetyloxy groups. Complete analysis of the spectrum established the structure of the diacetate as 5'-*O*-(4-chlorobenzoyl)-2', 3'-di-*O*-acetyluridine (**12**).

Thus, regioselective cinnamoylation at 3' position by dibutyltin oxide mehtod and 5' position by direct method have been observed. Similarly, 3–bromobenzoylation and 4-chlorobenzoylation at 5' position by direct mehtod have also been observed.

Antibacterial activities

Besides synthesis, a comparative study on antibacterial activities of uridine and some of its acylated derivatives (Fig.-1)

were carried out by the disc diffusion mehtod [9]. Synthesis of compounds **5-7** and compounds **9-11** have already been reported [8] and synthesis of rest of the compounds have been described in the synthetic part of this paper.

From Table–1 it is clear that most of the acylated derivatives of uridine were unable to show inhibition against *Bacillus subtilis,* though uridine itself showed marked inhibition (more than 28 mm). Again, diameter of zone of inhibition of all the acylated derivatives of uridine were very lower against *Salmonella typhi* as compared to that of uridine. *Eschericia coli*, another human pathogen was inhibited by compounds **3, 8, 9** and **12** but to some lower extent than that of uridine (more than 33 mm). So, the selectively acylated uridine derivatives (**3–12**) showed comparatively lower antibacterial functionality than uridine (**1**) against all the tested organisms. However, these derivatives of uridine may also be investigated against other micro-organisms and such a project is being in progress and will be reported later.

TABLE-1 INHIBITION OF TEST CHEMICALS AGAINST THE MICRO-ORGANISMS

Compo-und no.	% of chemicals used (20µg/disc)	Diameter of zone of inhibition in mm		
		Bacillus subtilis	*Salmonella typhi*	*Eschericia coli*
1	1.0	*28.00	*38.00	*33.00
	0.5	*29.00	*39.00	*33.45
3	1.0	--	--	16.00
	0.5	--	--	15.90
4	1.0	--	--	--
	0.5	--	--	--
5	1.0	--	--	--
	0.5	--	--	--
6	1.0	10.50	--	--
	0.5	11.25	--	--
7	1.0	--	--	--
	0.5	--	--	--
8	1.0	--	10.50	21.00
	0.5	--	11.30	22.30
9	1.0	21.25	19.25	15.75
	0.5	20.55	19.55	15.80
10	1.0	--	--	--
	0.5	--	--	--
11	1.0	--	18.50	--
	0.5	--	19.25	--
12	1.0	10.50	--	10.50
	0.5	12.50	--	11.20

["*" indicates marked inhibition and "--" means no inhibition]

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