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SYNTHESIS OF SOME BENZOXAZOLONE GLYCOSIDES

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Benzoxazolone glycosides, containing ribofuranosyl 2'-deoxyribofuranosyl, galactopyranosyl and glucopyranosyl moities have been synthesised by condensation of silylated benzoxazolones with appropriately substituted sugars using stannic chloride in dichloroethane. The structures and anomeric configurations of these new glycosides have been assigned using ^{1}H-NMR spectroscopy.

Key words: Benzoxazolone, Glycosides, NMR

Introduction

Biologically active nucleoside analogues whether prepared synthetically or isolated from natural sources have found clinical application in anti-viral and anti-cancer chemotherapy. Those obtained from natural sources and used clinically in the treatment of certain types of malignant diseases include Actinomycin D, Mithramycin, Bleomycin and Doxrubicin. Certain synthetic purine and pyrimidine derivatives like 6-mercaptopurine, thioguanine flourouracil and the nucleoside analogues such as arabinocytosine and the recently discovered broad spectrum anti-viral agent, ribavirin [1] have also found clinical applications and are drugs of choice for certain types of cancers. Manymore nucleoside analogues although not in clinical use as yet but have exhibited interesting biological properties include certain ribavirin analogues [2], formycin A and 2'-deoxyformycin A[3,4], 6-azacadeguomycin [5] and cadeguomycin [6,7,8]. Synthesis of new nucleoside analogues with possible biological activity is a fruitfull field of investigations and therefore synthesis of benzoxazolone nucleoside analogues was undertaken. The results of these studies are being described in this publication.

Materials and and Methods

Melting points are uncorrected. ^{1}H-NMR spectra were determined on a Bruker AM-300 NMR spectrometer in $CDCl_3$ or D_2O. Positive ion FAB and FD mass spectra were determined on Varian MAT 312 connected to MAT 188 data system and PDP 11/34 computer system. Optical rotations were recorded on Polartronic D-21 polarimeter.T. TLC was carried out on precoated silica gel sheets (E.Merck). The spots were visualised by u.v. light and also by iodine vapours.

Trimethylsilybenzoxazolone (1). Benzoxazolone[9] (200 mg), hexamethyldisilazane (4 ml) and trimethylchlorosilane (0.3 ml) were refluxed under exclusion of moisture for 2 hours. TLC using chloroform-ethylacetate (8:2) v/v as the developing solvent showed a single faster moving spot of trimethylsilyl benzoxazolone. The excess of hexamethyl disilazane was removed in vacuo and further condensations were carried out without isolating the trimethylsilyl benzoxazolone.

N(2',3',5'-tri-O-benzoyl-β–D-ribofuranosyl) benzoxazolone (2). Freshly prepared **1** (200 mg) was dissolved in 1,2-dichloroethane (3 ml). I-O-acetyl-2',3',5'-tri-O-benzoyl-β-D-ribofuranose (500 mg) and anhydrous stannic chloride (0.07 ml) were added and the solution was refluxed for 2 hours. It was then slowly poured into a stirred 5% $NaHCO_3$ solution (30 ml). Chloroform (30 ml) was added and the stirring was continued for further 30 minutes. The mixture was filtered through celite, organic layer was separated, the aqueous layer was extracted with chloroform (2x20 ml) and the combined chloroform extract was dried over Na_2SO_4. Evaporation of the solvent gave a syrup. Chromatography on silica gel column using chloroform-ethylacetate (4:1 v/v) as eluent afford **2** , as a gum which on recrystallisation form methanol afford needles, m.p. 152° in 42%yield. ^{1}H-NMR, ($CDCl_3$) exhibited signals at δ 4.7 (dd, 2H, J=2.88, 7.65Hz, H-5'),4.8 (dd, 1H, J=2.94, 7.2Hz, H-4'), 6.05 (t, 1H, H-3'), 6.1 (t, 1H, H-2'), 6.2 (d, 1H, J=4. 6Hz, H-1').

N(β–D-ribofuranosyl) benzoxazolone (3). The protected nucleoside **2** (40 mg) was dissolved in methanol containing 10% sodium methoxide (5 ml) and the solution was refluxed for 4 hours. Methanol was evaporated under reduced pressure, the residue was dissolved in distilled water was passed through Amberlite IR-120 resin in the H^+ form. The eluate was evaporated in vacuo to afford **3** as an amporphous solid (15mg), [α] D^{20} +22° (e, 1.02, CH_3 OH). ^{1}H-NMR in D_2O exhibited signals as δ 5.96 (d, 1H, J=4. 75Hz, H-1'). FD mass showed M^+ at M/z 267. The high resolution mass spectrum exhibited exact mass at m/z 297.0848 (calcd. 297.08498) corresponding to molecular formula $C_{12}H_{13}NO_6$.

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N(3', 5'-di-O-acetyl-2'deoxy-β–D-ribofuranosyl) benzoxazolone (**4**). To freshly prepared (**1**) (200mg) in dichloroethane (3ml) was added per-O-acetyl-2'-deoxy ribofuranose [10] (450mg) and anhydrous stannic chloride (0.08ml). The mixture was refluxed for four hours and then poured into stirred 5% $NaHCO_3$ solution (30ml). Addition of chloroform (40ml), stirring for further 1/2 hour and filtration over celite afforded the chloroform layer. The combined chloroform layers after extraction of aqueous phase with chloroform (2x20ml) were dried over Na_2SO_4 and evaporated to dryness in vacuo. Separation on a silica gel column afforded two products, corresponding to Rf 0.05 and O.11 on TLC in solvent chloroform-ethylacetate (9:1 v/v). The product corresponding to Rf 0.05 was obtained as an amorphous solid in 30% yield and was benzoxazolone (**4**) ^{1}H-NMR $CDCl_3$, exhibited signals at δ 2.11 - 2.57 (m, 2H, H-2'a, H-2'b) δ 5.91 (t, 1H, H-1').

N(3',5'-di-O-acetyl-2'-deoxy-α-D-ribofuranosyl) benzoxazolone (5). The compound Rf 0.11 was obtained in 20% yield, recrystallisation from methanol afforded N(3',5'-di-O-acetyl-2'-deoxy-α-D-ribofuranosyl) benzoxazolone **5** as needles, m.p. 144°. ^{1}H-NMR, $CDCl_3$ exhibited signals at δ 1.94 - 2.95 (m,2H, H-2'a, H-2'b), 6.01 (dd, 1H, J=2 4Hz and 7.8Hz, H-1').

N (2'deoxy-β-D-ribofuranosyl) benzoxazolone (6). Deacetylation of **4** using 10% sodium methoxide/methanol and following the procedure as for compound **3** afforded the deblocked deoxyriboside **6** in 32% yield as amorphous solid [α] D^{20}+72° (e, 0.05, MeOH). ^{1}H-NMR, D_2O showed anomeric proton at δ 5.90 (t, 1H, H-1'). Positive ion FAB *m/z* 274 (M^+ + Na, 40%), 252 (M^+ + H, 31%), high resolution mass spectrum m/z 252.0873 (calcd. 252.08719) corresponded to melecular formula $C_{12}H_{14}NO_5$.

N(2'-deoxy-α-D-ribofuranosyl) benzoxazolone (**7**). Deacetylation of **5** using sodium methoxide in methanol afforded the deblocked isomer **7** in 23% yield as amorphous solid [α] D^{20} -60° (e, 0.05, MeOH). ^{1}H-NMR, D_2O displayed signal at δ 5.94 (dd, ^{1}H, J=3.0, 7.8 Hz, H-1'). Positive FAB m/z 274 (M^+ + Na, 28%), 252 (M^+ + H, 26%), high resolution mass spectrum *m/z* 252.0871 (calcd. 252.08719) gave molecular formula $C_{12}H_{14}NO_5$

N(2',3',4',6'-tetra-O-acetyl-β–D-galactopyranosyl) benzoxazolone (8). Condensation of **1** (200mg) with per-O-acetyl galactopyranose [11] (450mg) using stannic chloride (0.14ml) in dichloroethane (4ml) was carried out by refluxing for 12 hours. Purification of the product on silica gel column using chloroform hexane (4:1 v/v) gave the pure compound as amorphous solid in 70% yield. ^{1}H-NMR spectrum $CDCl_3$ exhibited signals at δ 2.16, 2.06, 2.02 and 1.90 (m, 12H,-O-acetyl), 5.92(d, 1-H, J=8.5Hz, H-1') 4.99 (dd, 1-H, J=9.OHz and 10.5Hz, H-2), 4.63 (m, 1H, H-3), 3.96 (m, 2H, CH_2-6'),4-45 (m, 1H, H-5').

N(β–D-galactopyranosyl) benzoxazolone (9). Deacetylation of **9** using 10% sodium methoxide/methanol followed by usual workup procedure as used for (**3**) afforded **9**. Re-crystallisation from methanol gave needles, m.p. 165° in 60% yield [α] D^{20}+81° (e, 0.05 MeOH). FD mass exhibited M^+ at *m/z* 297 (100%), high resolution mass spectrum *m/z* 297.0847 (calcd. 297.0849) attributable to the molecular formula $C_{13}H_{15}NO_7$.

N(2',3',4',6'-tetra-O-acetyl-β-D-glucopyranosyl benzoxazolone (***10***). Freshly prepared **1** (200mg) was allowed to react by refluxing for 12 hours with per-O-acetyl glucose [11] (400mg) in dichloroethane (4.5ml) using stannic chloride (0.15ml). The crude reaction mixture after usual workup was separated, on a silica gel column and **10** was obtained in 59% yield as amorphous solid using chloroform-hexane (9:1 v/v) as the eluent. ^{1}H-NMR, $CDCl_3$ showed signals at δ 1.92, 1.96, 2.02 (m, 12H, O-acetyl), 4.65 (m, 1H, H-5'), 4.21(m, 1H, H-6'), 4.65 (q, 1H, J=10Hz, H-3'), 5.15(t, 1H, H-4'), 5.66(t, 1H, H-2'), 6.08 (d, 1H, J=8, 5Hz H-1').

N(β-D-glucopyranosyl) benzoxazolone (***11***). De-O-acetylation of **10** with 10% sodium methoxide/methanol following the procedure as for compound (**3**) afforded the deblocked nucleoside **11**, recrystallisation from methanol afforded needles in 52% yield, m.p. 220°[α] D^{20}+32° (e, 0.75, CH_3OH). FD mass spectrum exhibited M^+ at *m/z* 297 (100%), high resolution mass spectrum *m/z* 297.0848 (calcd. 297.0849) gave molecular formula $C_{13}H_{15}NO_7$.

Results and Discussions

A literature survey indicated that for the preparation of nucleosides a versatile and convenient method is available which involves the glycosidation of peracetylated sugars with the tri-methylsilyl derivative of the base using stannic chloride as a catalyst. This method apart from being stereospecific is also specific for N-glycosidations [12,13] and hence was chosen for the glycosidations of benzoxazolone. The trimethylsilyl derivative of benzoxazolone **1** was prepared by refluxing benzoxazolone in hexamethyldisilazane and subsequent removal of the excess hexamethyl-disilazane by evaporation *in vacuo*. The condensation of **1** in situ with 1-O-acetyl-2, 3, 5-tri-O-benzoyl-β-D-ribofuranose using stannic chloride in dichloroethane resulted into the formation of a new product which was isolated by silica gel column chromatography in 40% yield using chloroform, ethyl acetate (9.5 :0.05 v/v) as the eluent. Structural elucidation by ^{1}H-NMR and by chemical methods confirmed the structure of the products as N (2',3',5'-tri-O-benzoyl-β-D-ribofuranosyl) benzoxazolone (**2**). The ^{1}H-

NMR in $CDCl_3$ exhibited the anomeric proton H-1' in **2** as a sharp doublet at δ 6.2($J_{1,2}$=4.6Hz) indicated a β glycosidic linkage [14]. The ribose H-2' and H-3' resonated as pseudotriplets at δ 6.12 and δ 6.65 respectively whereas H-4' and H-5' were present as doublets of doublet at δ 4.81 (J, 2.94 and 7.2Hz) and δ 4.72 (J, 2.88 and 7.65Hz) respectively. The formation of N-glycosydic bond was also confirmed chemically, the compound was stable in 2N acetic acid on heating, the O-glycosydic bond on the other hand is unstable under these conditions [14].

The debenzoylation of **2** with 10% sodium methoxide in methanol [15] afforded the unblocked N (β-D-ribofuranosyl) benzoxazolone **3** as an amorphous solid. ^{1}H-NMR spectrum in D_2O exhibited the anomeric H-1' at δ 5.96 as sharp doublet (J, 4.7Hz) indicating-β-glycosydic linkage. FD mass spectrum, showed M^+ at *m/z* 267, while high resolution mass spectrum exhibited the exact mass *m/z* 267.0741 (calcd. 267.0743) which corresponded with the molecular formula $C_{12}H_{13}NO_6$.

The benzoxazolone **1** was then condensed with 1',3',5'-tri-O-acetyl-2'-deoxyribofuranose using stannic chloride in dichloroethane. Separation of the reaction mixture on a silica gel column using a mixture of chloroform ethylacetate (9:1 v/v) as the eluent gave two products which were characterised as N(3',5'-di-O-acetyl-β-D-2'-deoxyribofuranosyl) benzoxazolone **4** and its anomer N(3',5'-di-O-acetyl-α-D-ribofuranosyl) benzoxazolone **5** by using ^{1}H-NMR spectroscopy. The ^{1}H-NMR spectra of **4** in $CDCl_3$ exhibited the H-1' signal as a triplet at δ 5.91, (J=6.7Hz) while in the α-isomer **5** it appeared as doublet of doublets at δ 6.01, (J=2.4 and 7.8Hz). On the basis of these signals, the compound **4** and **5** are assigned the β and α isomers of deoxyribosylated benzoxazolone respectively [16]. The de-O-acetylation of **4** and **5** was carried out using sodium methoxide in methanol to afford the corresponding isomers **6** and **7** respectively as amorphous solids. The ^{1}H-NMR spectra of **6** in D_2O showed a triplet at δ 5.90 (J=6.2Hz) confirming this compound as N(β-D-2'-deoxyribofuranosyl) benzoxazolone **6.** The ^{1}H-NMR spectrum of **7** in D_2O exhibited the H-1' as doublet of doublets at δ 5.94 (J=3.0 and 7.8Hz), which indicates this compound as N (α-D-2'-deoxyribofuranosyl) benzoxazolone (**7**). Furthermore it has been known [17] that the difference in chemical shift of two methylene protons at C-2' of α -deoxyriboside is bigger than that of β-isomer. Our results also show that the signal of H-2' a and H-2'b for **7** appeared as a set of separate multiplets between δ 1.94 and δ 2.95 whereas for **6** a clustered multiplets is observed between δ 2.11 and δ 2.57, this observation is consistent with the assigned structures of the two anomers [17]. Positive ion FAB mass spectrum of **6** and **7** showed $(M^++H)^+$ at *m/z* 252, and $(M^++Na)^+$ at *m/z* 274, while high resolution masss spectrum gave exact mass at *m/z* 252.0873 (calcd. 252.08719) attributable to the molecular formula $C_{12}N_{14}HO_5$.

The stannic chloride catalysed glycosylation of **1** was carried out with 1,2,3,4,6-penta-O-acetyl-β-D-galacto-pyranose, the reaction took a longer time, i.e. 12 hours refluxing of the reaction mixture. The galactosylated benzoxazolone, N (2',3'4',6'-tetra-*O*-acetyl-β-D-galacto-pyranosyl) benzoxazolone (**8**) was purified by silica gel column chromatography using chloroform-hexane (4:1 v/v) as the eluent.

The ^{1}H-NMR spectrum of **8** in $CDCl_3$ exhibited the acetate methyls at δ 2.16, 2.06 and 1.90 integrating for 12 protons. According to the acetyl resonance rule [18] hexopyranosyl nucleosides with axial O-acetyl groups give a larger separation of signals hence the distinct low field resonance at δ 2. 16 is clearly attributable to the axial 4'-acetoxy group. The anomeric H-1' is found as a doublet at δ 5.92 and (J=8. 5Hz) indicating a β-glycosidic linkage. The H-2' resonated as doublets of doublet at δ 4.99 (J=9.0 and 10.5Hz) and H-3' is located as a multiplet at δ 4.63. The methylene protons at C-6 were exhibited as a multiplet at δ 3.96 integrating for 2 protons. The H-5' resonated as a multiplet of ABX system at δ 4.45.

De-O-acetylation of **8** with methanolic sodium methoxide gave the deprotected N (β-D-galactopyranosyl) benzoxazolone (**9**) as a crystalline solid. The FD mass spectrum of **9** exhibited the M^+ at *m/z* 297, while high resolution mass spectrum gave exact mass at *m/z* 297.0847 (calcd. 297.0849) corresponding to the molecular formula $C_{13}H_{15}NO_7$.

The stannic chloride catalysed condensation of **1** with 1,2,3,4,6-penta-O-acetyl-β-D-glucose by refluxing for 12 hours in dichloroethane gave N(2',3,',4',6',-tetra-O-acetyl-β-D-glucopyranosyl) benzoxazolone (**10**) which was subsequently purified by silica gel column chromatography. The ^{1}H-NMR spectrum of the product in $CDCl_3$ exhibited acetate methyls at δ 2.02 integrating for 6 protons and at δ 1.96 and δ 1.92 each integrated for 3 protons. As expected the glucopyranosyl benzoxazolone did not exhibit a signal attributable to an axially oriented acetoxy group as was observed in the galacto-pyranosyl benzoxazolone **8.** The anomeric signal was a doublet at δ 6.08 (J=8.7Hz) indicating a β-glycosyl linkage. The H-2' and H-4' resonated as triplets at δ 5.66 and δ 5.15 respectively. The H-3' appeared as a quartet of 10Hz at δ 4.65 whereas H-5' and methylene protons of C-6 resonated as multiplets at δ 4.05 and δ 4.21 respectively.

De-blocking of **10** using sodium methoxide in methanol afforded the unprotected glycoside N(β-D-glucopyranosyl)

benzoxazolone 11 as a crystalline solid. The FD masss spectrum exhibited the M^+ at *m/z* 297 and high resolution mass spectrum gave exact mass at *m/z* 297.0848 (calcd. 297.08498) corresponding to the molecular formula $C_{13}H_{15}NO_7$.

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