Phase Transfer Synthesis of Sulphamethoxazole N^1(methyl 2,3,4-tri-O-acetyl-β-D-glucuronide)

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Summary: Sulphamethoxyazole-N^1-(methyl 2,3,4-tri-O-acetyl-β-D-glucuronide) was successfully synthesized and characterized by NMR and mass spectrometry for the metabolic study of sulphamethoxazole.

Introduction

Glucuronides covalent conjugates of β-D-glucopyranosiduronic acid, are formed in the Phase-II metabolism of most drugs, environmental contaminants and other xenobiotics that make their way into the human body. Their aqueous solubility makes them easily excretable in the uring [1].

Sulphamethoxazole and trimethoprim are each mainly bacteriostatic when used alone but when combined (5:1 ratio) (Co-trimoxazole, Bactrim, Septrin), they become an antibacterial agent [2,3]. Metabolic excretion of N^1 and Ring-N, glucuronides of sulphamethoxazole has been reported [4], while N^1 and N^4-glucuronides of other sulphonamides have also been reported [5-16], but the evidence for the precise structure of the proposed conjugates has not been supported by the modern analytical techniques like NMR and mass spectrometry. The major problem seems to be the difficulty of synthesizing such required authentic conjugates in a pure state. The present article describes the synthesis of the N^1-β-D-glucuronide (protected) of sulphamethoxazole by an adaptation of the Dess, et. al. [17] method, and its characterization by proton NMR and mass spectrometry (EI).

Experimental

Sulphamethoxazole-N^1-(methyl 2,3,4-tri-O-acetyl-β-D-glucuronide) (I) was synthesized, using the method of

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Dess, et. al. [17], by dissolving sulphmethoxazole (0.51g, 0.002 mole) and benzyltriethylammonium bromide (0.2 g, 0.001 mole) in aqueous sodium hydroxide (1.25M, 2 ml). The resulting solution was added to a solution of methyl 2,3,4,-tri-O-acetyl-α-bromoglucuronate (0.41g, 0.001 mole) in chloroform (5 ml). The resulting mixture was stirred vigorously and heated under relux (3h). Aftr cooling, water (5 ml) was added. The chloroform layer was separated and washed twice with aqueous sodium hydroxide (1.25 M, 3ml) and dried (sodium sulphate). The solvent was removed yielding a yellow amorphous solid. Recrystallization from ethanol yielded the product. (0.73 g, 64%), m.p. 112-114°. Found: C, 48.6; H, 4.7; N, 7.3. $C_{23}H_{27}O_{12}S$ requires: C, 48.5; H, 4.7; N, 7.4%. NMR (proton) recorded at 80 MHz on a Bruker model WP80 SY spectrometer gave the following data: δ ($CDCl_3$): 1.95 (3H, s): 2.0 (3H, s,); 2.02 (3H, s); 2.4 (3H, s); 3.8 (3H, s); 4.1 (1H, d, J=9Hz); 4.9-5.3 (3H, m); 5.6 (1H, d, J=9Hz); 6.1 (1H, s); 6.6 (2H, d, J=10Hz); 7.6 (2H, d, J=10Hz). Mass spectrum (EI) recorded with VG-Analytical ZAB-IF, gave the following data: m/z (%): 570 (M+1,40), 505 (M-SO_2, 85), 488 (5), 462 (570-2 OAc, 27), 446 (505-OAC, 27), 408 (488-OAc, 17); 317 (5), 257 (9), 215 (7); 189 (7), 174 (13), 156 (47), 155 (60), 127 (43), 108 (28), 92 (45), 65 (20), 55 (4), 43 (Ac, 100).

N^4-Acetyl SUlphamethoxazole-N^1-(methyl 2,3,4-tri-O-acetyl-β-D-glucuronide) (II) was prepared by dissolving sulphamethoxazole-N^1-(methyl 2,3,4-tri-O-acetyl- β -D-glucuronide) (I) (0.03g) in acetic anhydride (1 ml) and the solution was heated under reflux (1h) with vigorous stirring. The contents of the flask were poured into the ice cold water yielding a colourless amorphous solid. Recrystallization from ethanol yielded the product (38 mg, 60%), m.p. 123-125°. Found: C, 48.8; H,4.9; N, 6.6 $C_{25}H_{29}O_3O_{13}S$ requires: C, 49.1; H, 4.7; N, 6.8%. NMR (proton) δ($CDCl_3$): 1.9 (3H, s); 1.95 (3H, s); 2.05 (3H, s); 2.15 (3H, s); 2.4 (3H, s); 3.8 (3H, s); 4.1 (1H, d, J=9Hz); 4.85-5.3 (3H, m); 5.7 (1H, d, J=9Hz); 6.1 (1H, s,); 7.85 (4H, d, J=5Hz). Mass spectrum (EI) m/z (%): 612 (M+1,2), 552 (M-OAc,3), 504 (2), 493 (M-2 OAc, 4), 488 (552-SO_2, 7), 317 (5), 296 (12), 257 (6), 231 (7), 216 (7), 193 (28), 181 (8), 156 (18), 155 (44), 140 (31), 134 (29), 127 (33), 108 (25), 92 (26), 83 (8), 65 (29), 60 (63), 43 (Ac 100).

Discussion

The previous methods used for the synthesis of protected glucuronides [18-22] have generally resulted in rather low yields of the desired conjugates. The real advantage of the adopted Dess method [17] is that it involves a phase transfer technique in which the protected bromoglucuronide is held in the organic phase (chloroform), and in this way is somewhat protected from premature attack by the alkali. The sulphamethoxazole is dissolved in a slight excess of aqueous alkali, thus generating-SO_2-N^- -ion; this will then attack, nucleophilically, the bromoglucuronate (protected) when the two are brought into intimate contact by the influence of the benzyltriethylammonium bromide (a phase transfer catalyst) at the interface. The reaction product will be lipophilic and so will be distributed predominantly in the organic phase and will consequently be protected from hydrolytic breakdown by the residual alkali. Thus this simple method was found to give excellent yields of the required protected glucuronide conjugate.

The linkage of the protected glucuronide moeity was shown to be to the sulphonamido nitrogen (N^1) atom of the sulphamethoxazole by acetylating the protected conjugate, and analysing the resulting product by proton NMR. There was a clear downfield shift of the upfield aromatic doublet from δ 6.6 to δ 7.85, as would be expected to result from the acetylation of an amino ($-NH_2$) group linked directly to an aromatic ring rather than the amido group. Had the protected glucuronide been linked to the amino ($-NH_2$) nitrogen, and the amide nitrogen (SO_2-NH-) undergone acetylation, the resulting proton NMR changes would not have accorded with our observations.

The stereochemistry of the linkage at the glucuronide (protected) would be expected to be β, if the attack by the sulphonamide anion on the protected bromoglucuronide had followed the SN^2 mechanism. That the link was actually β, was supported by the NMR analysis of the anomeric proton; which showed a doublet at δ 5.6 with J = 9Hz; these values are consisten with the expected β-glucuronide linkage [18].

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