

Phase Transfer Synthesis of Sulphamerazine-N¹- (methyl 2,3,4-tri-O-acetyl-β-D-glucuronide)

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Abstract □ Sulphamerazine-N¹-(methyl 2,3,4-tri-O-acetyl-β-D-glucuronide) was successfully synthesized and characterized by proton NMR and mass spectrometry for the metabolic study of sulphamerazine.

Keywords □ Sulphamerazine, N-glucuronide (protected), Phase Transfer Synthesis.

Glucuronides, covalent conjugates of β-D-glucopyranosiduronic acid, are formed in the phase-II metabolism of drugs, environmental contaminants and other xenobiotics that make their way into the human body.¹⁻⁵ The aqueous solubility of the glucuronide conjugates makes them easily excretable in the urine.¹⁾

N¹ and N⁴-glucuronides of sulphamerazine and other sulphonamides have been reported²⁻¹⁰ but proposed structures of the conjugates have not been authenticated because of the difficulty in synthesizing such required authentic conjugates in a pure state. The present article describes successful synthesis of the N¹-β-D-glucuronide (protected) of sulphamerazine by the method of Dess *et al.*¹¹⁾ and its characterization by using proton NMR and mass spectrometry with electron impact mode of ionization.

EXPERIMENTAL METHODS

Sulphamerazine-N¹-(methyl 2,3,4-tri-O-acetyl-β-D-glucuronide (I) was synthesized, using the method of Dess *et al.*¹¹⁾ Sulphamerazine (0.55g, 0.002 mole) and benzyltriethylammonium bromide (0.28g, 0.001 mole) were dissolved in aqueous sodium hydroxide (1.25M, 2 ml). The resulting solution was then added to a solution of methyl 2,3,4-tri-O-acetyl-β-bromoglucuronate (0.41g, 0.001 mole) in chloroform (5 ml). The mixture was stirred vigorously and heated under reflux (3h). After cooling, water (5ml) was added. The chloro-

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form layer was separated and washed twice with aqueous sodium hydroxide (1.25M, 3ml) and dried (sodium sulphate). The solvent was removed yielding a yellow amorphous solid. Recrystallization from ethanol yielded the product. (0.68g, 62%), m.p. 212-214 °.

N⁴-Acetyl sulphamerazine-N¹-(methyl 2,3,4-tri-O-acetyl-β-D-glucuronide) (II) was prepared by dissolving sulphamerazine-N¹-(methyl 2,3,4-tri-O-acetyl-β-D-glucuronide) (I) (0.03g) in acetic anhydride (1ml) 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 dioxan-water (1:3) yielded the product (26mg, 85%), m.p. 211-214 °.

RESULTS

Microanalysis of the synthetic products was performed on a Carlo Erba Elemental Analyser model 1106 showing the following composition.

Compound (I): Anal Calcd. for C₂₄H₂₈N₄O₁₁S, C, 49.7; H, 4.9; N, 9.7%. Found: C, 49.4; H, 4.9; N, 9.5.

Compound (II): Anal Calcd. for C₂₆H₃₀N₄O₁₂S, C, 50.2; H, 4.9; N, 9.0%. Found: C, 49.7; H, 4.9; N, 8.8.

NMR (proton) recorded at 80MHz FT on a Bruker model WP80SY spectrometer gave the following data. Compound (I): δ (CDCl₃): 1.8 (3H,s); 2.0 (3H,s); 2.05 (3H,s); 2.4 (3H,s); 3.8 (3H,s); 4.0-4.5 (3H,m); 5.6 (1H,m); 6.1 (1H,d,J=9Hz); 6.6 (2H,d,J=10Hz); 6.8 (1H,d,J=5Hz);

7.93 (2H,d,J=10Hz); 8.35 (1H,d,J=5Hz). Compound (II): δ (CDCl₃): 1.85 (3H,s); 2.0 (3H,s); 2.05 (3H,s); 2.23 (3H,s); 2.4 (3H,s); 3.75 (3H,s); 4.17-4.35 (1H,m); 5.33-5.55 (2H,m); 5.95 (1H,d, J=9Hz); 6.23 (1H,d,J=9Hz); 6.83 (1H,d,J=5H); 7.57 (2H,d,J=10Hz); 8.1 (2H,d,J=10Hz); 8.33 (1H,d,J=5Hz).

Mass spectra (EI) recorded with VG-Analytical ZAB-1F, gave the following data. (I): m/z (%): 516 (M-SO₂,8); 458 (7), 457 (516-OAc,31),397 (457-OAc,7), 337 (397-OAc,21), 264 (7), 243 (6), 231 (7), 230 (47), 201 (17), 200 (38), 156 (29), 108 (18), 93 (12), 92 (40), 65 (10), 43 (Ac, 100). (II): m/z (%): 499 (M-SO₂-OAc, 9), 439 (499-OAc, 10), 307 (M # 1-Sugar, 10), 271 (36), 243 (12), 134 (17), 93 (21), 92 (14), 65 (17), 43 (Ac, 100).

DISCUSSION

The previous methods used for the synthesis of protected glucuronides have generally resulted in rather low yields of the desired conjugates. The advantage of the present method is that it involves a phase transfer technique by which the bromoglucuronide is held in the organic phase (chloroform), and in this way is somewhat protected from premature attack by the alkali. The sulphamerazine is dissolved in a slight excess of aqueous alkali, thus generating sulphonamido anion which will then attack, nucleophilically, the bromoglucuronide (protected) at the interface when the two are brought into intimate contact by the influence of the benzyltriethylammonium bromide (a phase transfer catalyst). 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 moiety to the sulphamido nitrogen (N¹) of the sulphamerazine was proven by acetylating the synthesized conjugate, and analysing the resulting product by proton NMR. There was a clear down-field shift of the aromatic doublet from δ 6.6 to 7.57, as would be expected to result from the acetylation of N⁴-amino group linked directly to an aromatic ring rather than amido group.

The stereochemistry of the linkage at the glucuronide (protected) was also determined to be β by NMR analysis of the anomeric proton; which showed a doublet at δ 6.1 with J=9Hz; these values are consistent with the expected β -glucuro-

nide linkage.¹²⁾

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