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Research Article

Synthesis of [$^2H_3$]-labelled sulfamethoxazole and its main urinary metabolites

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Abstract: Sulfamethoxazole was labelled at positions 3, 5, and 4′ by H/D exchange in a mixture of 5% $^2H_2SO_4$ and 95% ^2H_2O (v/v) under reflux for 72 h with good isotope incorporation and acceptable yield. Subsequently, [$^2H_3$]-sulfamethoxazole-N_1-glucuronide and [$^2H_3$]-N_4-acetyl-sulfamethoxazole were synthesized. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: sulfamethoxazole; deuterium label; sulfamethoxazole-N_1-glucuronide; N_4-acetyl-sulfamethoxazole

Introduction

Sulfamethoxazole (4-amino-N-(5-methylisoxazol-3-yl)-benzenesulfonamide) **(1)** is a chemotherapeutic widely used in the treatment of urinary and respiratory infections in humans as well as in agriculture. In the year 2003 in Germany more than 48 000 kg were prescribed for human medicine alone.[1] In humans **(1)** is extensively metabolized and excreted in the urine mainly as N_4-acetyl-sulfamethoxazole (N-(4-{[(5-methylisoxazol-3-yl)amino]sulfonyl}phenyl)-acetamide) (45–70%) and sulfamethoxazol-N_1-glucuronide (N-[(4-aminophenyl)- sulfonyl]-N-(5-methylisoxazol-3-yl)hexapyranuronosylamine) (5–15%).[2,3]

Highly sensitive mass spectrometric methods are used to investigate the environmental load of these substances and these assays require stable isotope internal standards.

Results and discussion

In principle, a *de novo* synthesis approach using deuterium-labelled acetanilide can be applied to prepare deuterium-labelled **(1)**. However, this requires a multi-step synthesis starting with an expensive precursor. Therefore, we decided to investigate acid catalyzed hydrogen–deuterium exchange reactions[4] followed by acetylation and glucuronidation, respectively (Figure 1). In an initial attempt we treated **(1)** with a mixture of 20% $^2H_2SO_4$ and 80% ^2H_2O (v/v) under reflux. H/D exchange and the stability of **(1)** was monitored by LC-MS (Figure 2). Whilst H/D exchange was rapid and almost exhaustive within 24 h, massive degradation took place and we were not able to isolate any product from the reaction mixture. To minimize this degradation the concentration of $^2H_2SO_4$ was reduced to 5% $^2H_2SO_4$ in 95% ^2H_2O (v/v). Under these conditions, after a reaction time of 72 h 38% of 3,5,4′-[$^2H_3$]-sulfamethoxazole **(2)** was isolated with an isotope incorporation of >93%. Prolonged reaction time even improved isotope incorporation. However, this resulted in a dramatic decrease in yield (Table 1). As microwave irradiation can be very useful in accelerating H/D exchange,[5] in an additional experiment, the H/D exchange reaction was carried out under microwave-enhanced conditions. After 1 h in a domestic microwave, at low energy to avoid release of vapor through the pressure control valve, the isotopic distribution was comparable to conventional heating under reflux for 50 h (Table 1). However, the reproducibility of results was unsatisfactory, possibly because of the inhomogeneity of the field in our domestic system.

(2) was converted to 3,5,4′-[$^2H_3$]-N_4-acetyl-sulfamethoxazole **(3)** by acetylation in pyridine according to Fujimoto and Okabe.[6] Glucuronidation was performed using 2,3,4-tri-O-acetyl-1-bromo-1-desoxy-α-D-glucopyranosiduronic acid methyl ester **(4)** and LiOH in

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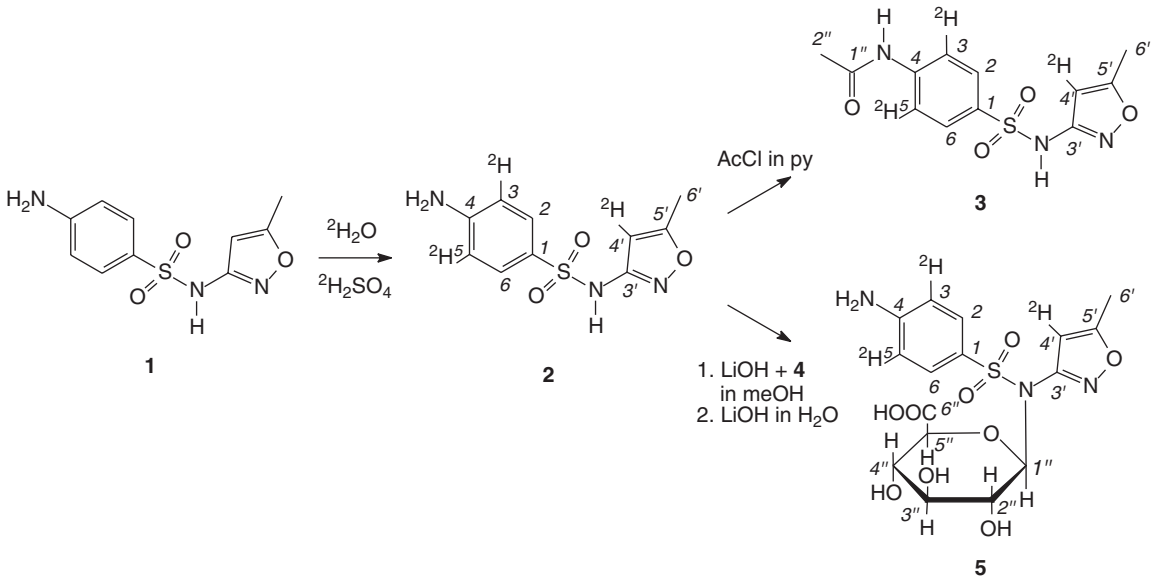

Figure 1 Synthesis of [$^2H_3$]-sulfamethoxazole, [$^2H_3$]-sulfamethoxazole-N_1-glucuronide and [$^2H_3$]-N_4-acetyl-sulfamethoxazole.

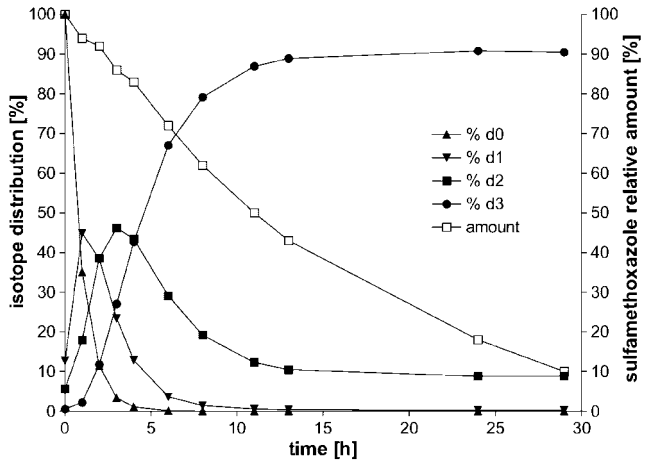

Figure 2 H/D exchange of sulfamethoxazole in 20% $^2H_2SO_4$ in 80% ^2H_2O (v/v). Reaction was performed as given in text. Samples were taken from the reaction mixture and immediately frozen and stored at −28°C until isotope distribution was determined by LC-MS. Quantification of total sulfamethoxazole in the reaction mixture was done by HPLC-UV at 254 nm. ▲: d_0; ▼: d_1; ■: d_2; ●: d_3; □: amount relative to t= 0 h.

methanol followed by deprotection with aqueous LiOH as described previously.[7] However, to achieve acceptable yields of 3,5,4′-[$^2H_3$]-sulfamethoxazol-N_1-glucuronide **(5)** elevated temperature and prolonged reaction times were necessary.

Experimental

Reactions requiring water-free conditions were performed in glass ware which was dried by heating under a stream of dry argon prior to use. All reactions were performed under an argon atmosphere. The degree of isotope labelling was determined by electrospray ionization-mass spectrometry (HP 1100, Agilent Technologies, Waldbronn, Germany) and calculated by comparison to the unlabelled compound. Identification of the localization of deuterium labels was done by comparison of ^{1}H-NMR spectra of labelled and unlabelled compounds at 250 or 500 MHz (Bruker, Karlsruhe, Germany).

3,5,4′-[$^2H_3$]-sulfamethoxazole (2)

Two gram of **(1)** was dissolved in 5 ml $^2H_2SO_4$ and 95 ml ^2H_2O and heated under reflux for 72 h. At the end of the reaction time the slightly yellow reaction mixture was cooled in an ice bath and the pH was adjusted to 5 with 5 M NaOH. During the adjustment of pH a white solid

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Table 1 H/D exchange of sulfamethoxazole in 5% $^2H_2SO_4$ in 95% ^2H_2O (v/v)

Time (h)	Isotope distribution (%)					Isolated yield (%)
	d_0	d_1	d_2	d_3	d_4	
48	0.00	0.83	30.2	69.0	0.06	62
72	0.00	0.09	6.45	93.32	0.10	38
96	0.00	0.00	3.1	96.7	0.23	13
60 min[a]	0.00	0.61	25.8	73.4	0.2	45

Note: Reactions were performed as given in text but terminated at the times indicated in the table.[a] Microwave.

precipitated. The reaction mixture was extracted three times with 100 ml ethyl acetate in which the precipitate dissolved immediately. The organic phase was dried over sodium sulfate and evaporated to dryness to obtain 753 mg (38%) of **(2)** as a white amorphous solid. Recrystallization from ethanol gave colorless cubic crystals. Yield: 659 mg (33% of theory). For isotope distribution see Table 1. Anal.: Calculated for $C_{10}H_8^2H_3N_3O_3S$: C, 46.86; H+^{2}H 4.33; N, 16.40; O, 18.73; S, 12.51. Found: C, 46.83; H+^{2}H 4.36; N, 16.43; S, 12.68. ^{13}C-NMR (in $C^2H_3O^2H$): 171.7 (C-5′); 159.6 (C-3′); 154.7 (C-4); 130.2 (C-2, C-6); 126.4 (C-1); 113.6–114.4 (m, low intensity, C-3, C-5); 95.8–96.7 (m, low intensity, C-4′); 12.3 (C-6′). ^{1}H-NMR (in $C^2H_3O^2H$): 7.58 (s 2H, C-2, C-6); 6.13 (s 0.04H, C-4′); 2.32 (s 3H, C-6′). (Assignment was done according to COSY experiments and in agreement with Saito *et al.*[8])

3,5,4′-[$^2H_3$]-sulfamethoxazole (2) microwave assisted

In a closed PTFE-reaction vessel equipped with a pressure control valve (approximately 7 bar) 500 mg of **(1)** was dissolved in 1.5 ml $^2H_2SO_4$ and 28.5 ml ^2H_2O. The reaction vessel was irradiated in a domestic microwave oven at a nominal energy of 80 W (1/10 of time with a fixed energy of 800 W) for 60 min. After cooling down to room temperature, the reaction vessel was opened, cooled in an ice bath and the product was separated as described above, to obtain 224 mg (45% of theory) as a white solid.

3,5,4′-[$^2H_3$]-N$_4$-acetyl-sulfamethoxazole (3)

To a solution of 256 mg (1 mmol) **(2)** in 5 ml dry pyridine, 142 µl (2 mmol) acetyl chloride was added. Spontaneously, a white solid precipitated. This precipitate re-dissolved while the reaction mixture was stirred at 100°C. After 30 min the yellow solution was cooled in an ice bath and diluted with 25 ml of ice-cold 1 M NaOH. Acidifying of the reaction mixture with chilled 10% hydrochloric acid resulted in precipitation of **(3)**. The precipitate was washed with diluted hydrochloric acid and water and dried to obtain 250 mg (84% of theory) of **(3)** as a slightly brown solid of a purity >99% (HPLC-UV at 254 nm). Anal.: Calculated for $C_1^2H_{10}^2H_3N_3O_4S$: C, 48.31; H+^{2}H, 4.40; N, 14.09; O, 21.45; S, 10.75. Found: C, 48.34; H+^{2}H, 4.41; N, 14.10; S, 10.94. Isotope distribution: d_0: 0.00, d_1: 0.07, d_2: 7.03, d_3: 93.1, d_4: 0.2. ^{13}C-NMR (in $^2H_6$-DMSO): 170.2 (C-5′); 169.1 (C-1″); 157.5 (C-3′); 143.4 (C-4); 132.8 (C-1); 127.9 (C-2, C-6); 118.4 (low intensity) (C-3, C-5); 95.3 (low intensity) (C-4′); 24.1 (C-2″); 12.0 (C-6′). ^{1}H-NMR (in $^2H_6$-DMSO): 7.79 2H (C-2, C-6); 6.1 0.05H (C-4′); 2.30 s 3H (C-6′); 2.10 s 3H (C-2″).

(4-amino-N-(β-D-glucopyranuronosyl)-N-(5-methyloxazol-3-yl)-benzenesulfonamide) (5)

To a hot solution of 550 mg of **(2)** in 7.5 ml methanol, 90.2 mg of LiOH and 854 mg of **(4)** were added and stirred under reflux for 90 min. After the reaction mixture was cooled in an ice bath, 496 mg of LiOH in 7.5 ml water was added and the solution was stirred for additional 30 min at room temperature. The pH of the reaction mixture was adjusted to pH 4 by the addition of glacial acetic acid and unchanged **(2)** was extracted three times with 15 ml chloroform. The organic phase was dried over sodium sulfate and evaporated to dryness to obtain 377 mg (68.5%) of **(2)** as a yellow solid which was identified as pure **(2)** by TLC and HPLC. The aqueous phase was evaporated to dryness under reduced pressure. The foamy residue was stored over potassium hydroxide under reduced pressure to remove traces of acetic acid. The majority of hydrophilic by-products were removed by a column chromatography on silica gel 60 with acetone:methanol 50:50 (v/v). Final purification was carried out by chromatography on a Lobar B column packed with Lichroprep RP 18 (40–64 µm) (Merck, Darmstadt, Germany) with a two step gradient. Firstly, some brown by-products were eluted with water:methanol 85:15 (v/v) then **(5)** was separated from an unidentified glucuronide with water:methanol 2:1 (v/v). Freeze drying of the pooled fractions containing **(5)** resulted in 123 mg (13.2% of theory) of a white solid. Isotope distribution: d_0: 0.00,

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d_1: 0.08, d_2: 6.12, d_3: 93.7, d_4: 0.04. ^{13}C-NMR (in ^2H_2O): 175.1 (C-6″); 173.2 (C-5′); 156.6 (C-3′); 153.0 (C-4); 130.2 (C-2, C-6); 123.8 (C-1); 114.3 (m, low intensity, C-3, C-5); 102.5 (m, low intensity, C-4′); 86.6 (C-1″); 78.8 (C-5″); 76.7 (C-3″); 71.4 (C-4″); 69.6 (C-2″); 11.9 (C6′). ^{1}H-NMR (in ^2H_2O): 7.45 (s, 2H, C-2, C-6); 6.13 (s, 0.05H, C-4′); 5.21 (d, 1H, C-1″); 3.69 (d, 1H, C-5″); 3.49 (t, 1H, C-3″); 3.30 (t, 1H, C-4″); 3.14 (t, 1H, C-2″); 2.29 (s, 3H, C-6′).

Conclusion

Acid catalyzed H/D exchange by heating under atmospheric pressure or microwave assisted under elevated pressure is a suitable method to obtain deuterium-labelled sulfamethoxazole with high isotope incorporation and acceptable yield. Labelling proved to be stable during subsequent conjugation reactions to 3,5,4′-[$^2H_3$]-N_4-acetyl-sulfamethoxazole and 3,5,4′-[$^2H_3$]- sulfamethoxazole-N_1-glucuronide, respectively.

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