SYNTHESIS OF 2-FURANYLMETHYL-α-^{2}H AND -^{3}H FUROSEMIDE

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SUMMARY

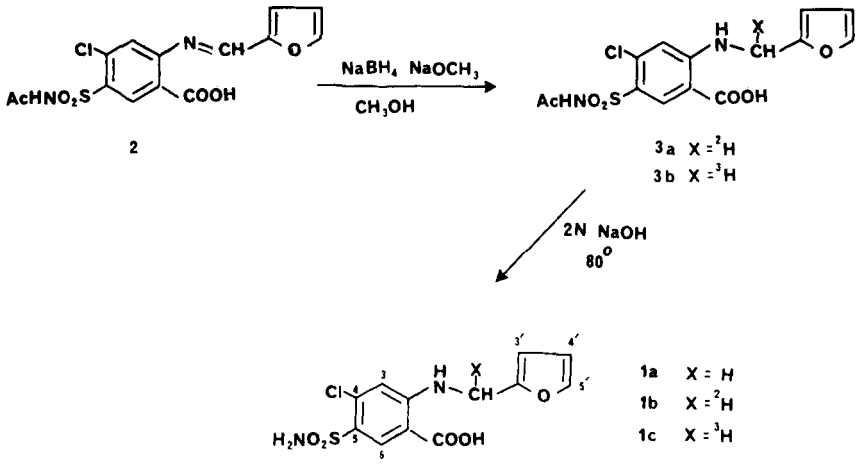
Synthesis of furosemide, specifically labelled at the 2-furanylmethyl α-position with ^2H or ^3H is reported. This synthesis required reduction of N-[(2-furanylmethyl)amino]-4-chloro-5-(N-acetylaminosulfonyl)benzoic acid (2) with sodium ^2H- or ^3H-borohydride, followed by alkaline hydrolysis of the acetyl group.

Introduction

Furosemide, 2-[(2-furanylmethyl)amino]-4-chloro-5-(aminosulfonyl)benzoic acid (1a), is an important diuretic used in the treatment of congestive heart failure and in renal insufficiency. Metabolic studies have been reported using ^{35}S-furosemide,[1] and with ^{3}H-furosemide prepared by catalytic exchange.[2,3] Recently, the preparation of carboxyl-^{14}C furosemide has been reported.[4] Metabolism experiments indicated the desirability for having available specifically labelled ^{2}H- and ^{3}H-furosemide. In this paper we report the successful synthesis of 2-furanylmethyl-α-^{2}H- and ^{3}H-labelled furosemide, (1b and 1c).

Discussion

Although furosemide is normally prepared by furfurylamine displacement of the 2-halide of a 2,4-dihalo-5-aminosulfonylbenzoic acid,[5,6] an alternate method has been reported involving the catalytic reduction of imine 2, which is formed by the reaction of 2-amino-4-chloro-5-(N-acetylaminosulfonyl)



benzoic acid with 2-furancarboxaldehyde.[7] Imine 2 was used as starting material for preparation of 1b and 1c.

Hydride reduction of 2 with sodium ^{2}H-borohydride, in methanol, provided 3a. Subsequent hydrolysis (aqueous 2N NaOH 80°) afforded 1b. The nmr spectrum (DMSO-D_2O) provided confirmation for the site of the deuterium atom. The signal of the 2-furanylmethyl α-protons in furosemide was observed as a slightly broadened singlet at 4.63 δ, width at half height ≈ 4 Hz. In 1b, this signal integrated for only one proton. The signals of furan protons H-3', H-4' and H-5' remained unchanged in intensity and multiplicity.

Using sodium ^{3}H-borohydride in a similar procedure afforded 1c. On a 0.45 mmole scale of sodium ^{3}H-borohydride (55 mCi/mmole), 1c was obtained in 25% yield (based on starting imine 2), after chromatographic purification with a specific activity of 9.32 mCi/mmole.

Experimental Section

Radioactivity determinations were performed using a Beckman Model LS-230 liquid scintillation spectrometer. Sample counts were corrected for quench by the internal standard method using ^{3}H-toluene or by using an external standard calibrated for ^{3}H. The preparative thin layer chromatography (tlc) plates used were 20 x 20 cm glass plates coated with a 2 mm thick layer of silica gel 60 F-254 (EM Reagents). Chromatography solvents were either analytical reagent

grade or distilled prior to use. Compounds were visualized using uv (254 nm) illumination. Radiochemical purity was determined on 5 x 20 cm aluminum plates coated with 0.25 mm thick silica gel using a Berthold 6000-1 Radiochromatogram Scanner to locate the radioactive compounds. UV determinations were performed using a Coleman Model 101 Hitachi UV-Vis Spectrophotometer. Nmr spectra were determined on a Varian-A-60A spectrometer using TMS as internal standard. Mass spectral data were obtained on a SRI-Biospect mass spectrometer operated in the EI mode.

2-[(2-Furanylmethyl-α-^{2}H)amino]-4-chloro-5-(aminosulfonyl)benzoic acid (1b).

The imine 2, 40 mg (0.105 mmole) was dissolved in a solution of 6 mg sodium methoxide and 5 ml CH_3OH. Sodium ^{2}H-borohydride (Stohler Isotope Chemicals-99% ^{2}H), 32 mg was then added in 4 mg portions at 30 minute intervals with stirring. An additional 28 mg of sodium ^{2}H-borohydride was then added all at once and the reaction mixture stirred at room temperature overnight. To the reaction mixture was added 5 ml of aqueous 4N NaOH and the mixture heated to reflux for 2 hrs. The solution was then cooled to -5° and carefully acidified with aqueous 1N HCl. The precipitate was removed by filtration and fractionally recrystallized from 50° EtOH (decolorized with Norite) to yield 12 mg (0.0364 mmole; 35%) of 1b, mp 207°d. Thin layer chromatography of 1b showed that it migrated with the same Rf as an authentic sample of furosemide in two solvent systems: EtOAc:CH_3OH:NH_4OH (65:25:10); Rf 0.47 and $CHCl_3$:CH_3OH:HOAc (89:6:5); Rf 0.53; Nmr (DMSO-d_6, D_2O) δ, 4.63 (s, 1, -CHD-NH, width at half height ≃ 4 Hz), 6.48 (d, 2, H-3' and H-4', J = 1 Hz), 7.20 (s, 1, H-3), 7.70 (t, 1, H-5', J = 1 Hz), 8.58 (s, 1, H-6). Furosemide gave an identical spectrum except the signal at 4.63 δ integrated for 2 protons. M/e 331 and 333 for $C_{12}H_{10}{}^{2}H_1{}^{35}ClN_2O_5S$ and $C_{12}H_{10}{}^{2}H_1{}^{37}ClN_2O_5S$.

2-[(2-Furanylmethyl-α-^{3}H)amino]-4-chloro-5-(aminosulfonyl)benzoic acid (1c).

To a solution of 6 mg of sodium methoxide in 3.0 ml of CH_3OH was added 37 mg (0.1 mmole) of the imine 2 and the resulting solution cooled to 0°. Sodium ^{3}H-borohydride, 16.8 mg; 0.45 mmole (New England Nuclear, 25 mCi/6.8 mg diluted

with 10 mg of carrier sodium borohydride), was then added in 4 mg portions over the course of 5 hrs. with stirring. To the reaction mixture was added 0.6 ml of 10 N NaOH and the resulting solution heated to reflux for 2 hrs. The reaction mixture was then concentrated in vacuo to 1 ml and applied to a preparative tlc plate and developed with EtOAc:CH_3OH:NH_4OH (65:25:10). The furosemide band (Rf 0.50) was scraped, eluted with CH_3OH (5 x 50 ml), concentrated in vacuo at 35° and dissolved in 3 ml of 0.1N NaOH. A 5 microliter sample of this solution was diluted to 5 ml with distilled H_2O and its UV absorption determined at 229 nm and 272 nm.[8] These absorptions were then compared with those obtained from a standard furosemide curve (1-10 µg/ml). The yield of ^{3}H-furosemide, 1c, was 8.2 mg (0.025 mmole; 25%). The specific activity was found to be 28.4 µCi/mg; 9.32 mCi/mmole. Radiopurity was > 99%.

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