SYNTHESIS OF TRITIATED BUMETANIDE

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Summary

Tritium labelled bumetanide of high specific activity (16 Ci/mmol) has been prepared in our laboratory by tris(triphenylphosphine) rhodium chloride catalyzed reduction of an olefinic precursor with carrier free tritium gas in benzene-ethanol (1:1). The product is labelled in the N-butyl side chain. Comparison of both heterogeneous palladium and homogeneous rhodium catalyzed deuterium reductions as a model for the tritiation revealed that the latter reduction was accompanied by considerably less label scrambling.

Key Words: bumetanide, 3-[N-(1-but-2-enyl)amino]-4-phenoxy-5-sulfamylbenzoic acid, tris(triphenylphosphine)-rhodium chloride catalysis, catalytic reduction, deuterium, tritium.

Introduction

In recent years it has become apparent that much of the ouabain-insensitive, passive ion movements through biological membranes, thought earlier to occur by diffusion, is actually mediated by complex coupled co-ion transport mechanisms. Among the properties of these transporters recognized thus far, are saturation kinetics, high temperature dependence and interdependence of the transported ions. The inhibition by the "loop" or "high ceiling" diuretics, furosemide or bumetanide is of general pharmacological interest because these substances not only effectively inhibit Na^+K^+ cotransport in the loop of Henle[1,2] and in other epithelial cells[3,4] but also in a variety of non-epithelial cells such as nucleated bird red cells[5,6] or enuclate human red cells.[7,8] While the biochemical nature of the molecules translocating Na^+K^+ and Cl^- is unknown, there are recent physiologic studies indicating complex synergistic interactions between furosemide or bumetanide and the ions to be transported.[9,10,11]

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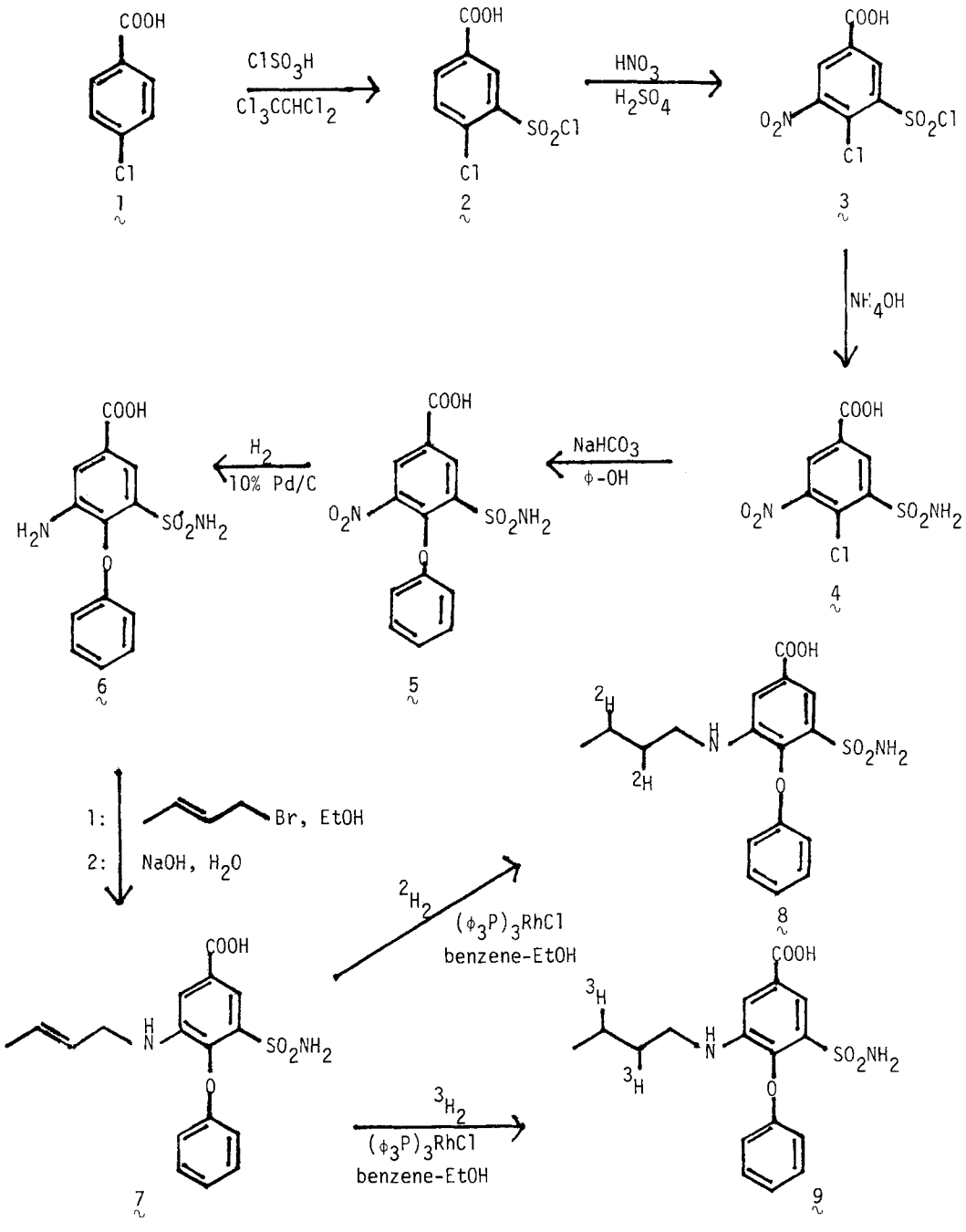
For a precise biochemical identification of the co-ion transport moieties as well as to determine their molecular transport activities and whether or not changes caused by evolutionary events or physiologic manipulations are of kinetic nature, radiolabelled compounds must be available. Recently, Forbush and Palfrey[12] have synthesized ^{3}H-bumetanide and ^{3}H-benzmetanide by reduction with $NaB^{3}H_4$ of the Schiff's base adduct between 3-amino-4-phenoxy-5-sulfamylbenzoic acid and butylaldehyde or benzaldehyde. The specific activities of the two loop diuretics synthesized by this procedure were in the range of 1-2.0 Ci/mmol, which is sufficient for labelling of membranes with high transport site density[12] but clearly too low for accurate site determination in membranes with low transport activities such as in red cells of birds[5,6] or of humans[7,8]. This situation is somewhat analogous to that of determining the number of $Na^{+}K^{+}$ pump molecules, where only relatively high specific activities have made such attempts successful in red cell membranes[13]. Here, we report the synthesis of bumetanide of higher specific activity (16 Ci/mmol) which is tritium labelled in the N-butyl side chain.

Discussion

It was felt that the most plausable precursor to tritiated bumetanide would be one in which a double bond might be reduced with carrier free tritium gas (Figure 1). Therefore, the preparation of 3-[N-(1-but-2-enyl)amino]-4-phenoxy-5-sulfamylbenzoic acid (7) was undertaken. Feit, et al.[14] has reported the synthesis of bumetanide and other derivatives in which the aryl amino substituent has been varied. The synthetic scheme employed is shown in Figure 1. Compounds 1-6 are each intermediates in the synthesis of unlabelled bumetanide and have been reported.[14,15,16] However, 7 is a novel compound and was prepared by the reaction of 6 with crotyl bromide in ethanol at reflux. Under these conditions, not only is the anilino nitrogen alkylated but the carboxylic acid is also converted to the ethyl ester. Upon alkylation and subsequent saponification, 7 was obtained in 28% yield from 6. This relatively low yield of 7 is in accord with results obtained by Feit[14] in most N-alkylations of 6. Initially, the preparation of 7 with a terminal double bond was attempted employing 4-bromo-1-butene instead of crotyl

Figure 1


COOH
Cl
1
ClSO3H
Cl3CCHCl2
SO2Cl
2
HNO3
H2SO4
O2N
3
NH4OH
4
NaHCO3
ϕ-OH
5
H2
10% Pd/C
H2N
SO2NH2
6
1:
2:
Br, EtOH
NaOH, H2O
7
2H2
(ϕ3P)3RhCl
benzene-EtOH
2H
8
3H2
3H
9

bromide whereupon no product was detected in the reaction mixture. Crotyl bromide, being an allylic bromide, offered a more reactive alkylating agent. Compound 7 was subsequently reduced catalytically with deuterium gas at 1.0 atm by two different procedures. Upon reduction of 7 using 10% Pd/C-THF, the deuterated product (8) was obtained in good yield after a 1.0 h reaction time with no evidence of 7 present by ^{1}H-NMR. Upon carrying out the reduction in benzene-ethanol (1:1) using the homogeneous catalyst, tris(triphenylphosphine)rhodium chloride, 7 was completely converted to 8 in 16 h. Palladium catalyzed deuterium and tritium reductions are ordinarily accompanied by scrambling of the label into other parts of the molecule whereas, this is usually less pronounced with the homogeneous catalyst.[17] Mass spectral analysis of the products from both reductions indicated that indeed the rhodium catalyzed reduction (d_0 = 3.13%, d_1 = 11.67%, d_2 = 69.11%, d_3 = 12.31%, d_4 = 2.96%, d_5 = 0.82%) was accompanied by considerably less scrambling than the palladium catalyzed reduction (d_0 = 18.48%, d_1 = 25.17%, d_2 = 24.32%, d_3 = 16.88%, d_4 = 9.65%, d_5 = 5.5%). We have routinely obtained d_2 values of >90% for double bond reductions catalyzed by tris(triphenylphosphine)rhodium chloride in benzene solution[18] for substrates without labile hydrogen (i.e., -OH, -NHR, -COOH, etc.). The lower value (69.11%) obtained here is most probably due to the presence of labile hydrogens attached to the -COOH, $-SO_2NH_2$ and -NHR groups of the substrates as well as the ethanol used to solubilize the substrate. Based upon the above results, the unsaturated precursor was tritiated under the identical conditions using the homogeneous rhodium catalyst to afford 352 mCi of product 9 with a specific activity of 16 Ci/mmol (43 mCi/mg).

Experimental Procedures

All chemicals were used as obtained from the manufacturer. Melting points were obtained on a Thomas Hoover Melting Point Apparatus and are uncorrected. ^{1}H-NMR spectra were obtained on a JEOL FX-60 60 MHz FT spectrometer using either $CDCl_3$, $(CD_3)_2CO$ or $(CD_3)_2SO$ (TMS) as solvent. Ultraviolet spectra were obtained on a Cary 15 UV spectrometer using methanol as solvent. Radiopurity was determined using a Packard Radioscanner Model 7201. Tritium was counted using a

Packard Liquid Scintillation Counter Model 3255 (internal standard) with Scintiverse R (Fisher) counting solution. Silica gel plates (UV) were used for TLC analyses. Elemental compositions of novel compounds were determined by high resolution mass spectrometry using an AEI MS-902 mass spectrometer.

3-Chlorosulfonyl-4-chlorobenzoic Acid (2). This compound was prepared by reaction of chlorosulfonic acid with p-chlorobenzoic acid (1) in pentachloroethane according to the procedure of Jackman, et al.[16] to afford the product (59%) as yellow crystals; mp 163-167°C (lit.[16] mp 168-170°C). ^{1}H-NMR ($CDCl_3$) (TMS) δ 8.86 (d, 1H, ArH), 8.30 (q, 1H, ArH), 7.80 (d, 1H, ArH).

3-Nitro-4-chloro-5-chlorosulfonylbenzoic Acid (3). The product was obtained by nitration of 2 with HNO_3/H_2SO_4 according to the procedure of Feit, et al.[15] to afford a colorless solid (85%); mp 177-182°C (crude) (lit.[15] mp 193-194°C after recrystallization). ^{1}H-NMR ($CDCl_3$) (TMS) δ 9.00 (d, 1H, ArH), 8.74 (d, 1H, ArH).

3-Nitro-4-chloro-5-sulfamylbenzoic Acid (4). Using the procedure of Feit, et al.[15], the product was obtained by reaction of 3 with conc. NH_4OH at room temperature to afford 4 (52%) as a yellow solid after recrystallization from methanol; mp 145-237°C (lit.[15] mp 235-236.5°C). ^{1}H-NMR ($(CD_3)_2SO$) (TMS) δ 8.71 (d, 1H, ArH), 8.43 (d, 1H, ArH), 7.53 (s, 2H, $-SO_2NH_2$).

3-Nitro-4-phenoxy-5-sulfamylbenzoic Acid (5). Using the procedure of Feit, et al.[14] the product was obtained by reaction of 4 with phenol in the presence of $NaHCO_3$ to afford a tan solid (14%) after column chromatography on silica gel 60 ($CHCl_3$-MEOH 9:1, 8:2); mp 250-252°C (lit.[14] mp 255-256°C). ^{1}H-NMR ($(CD_3)_2SO$) (TMS) δ 8.89 (d, 1H, ArH), 8.67 (d, 1H, ArH) 7.22 (m, 5H,-O-φ), 4.28 (s, 2H, $-SO_2NH_2$).

3-Amino-4-phenoxy-5-sulfamylbenzoic Acid (6). Using the procedure of Feit, et al.,[14] the nitro compound (5) as the lithium salt was reduced with H_2 in the presence of 10% Pd/C in water to afford a light tan solid (100%) which was not further purified; mp 249-251°C (lit.[14] mp 256-257°C). ^{1}H-NMR ($(CD_3)_2CO$) (TMS) δ 7.91 (d, 1H, ArH), 7.80 (d, 1H, ArH), 7.10 (m, 5H, -O-φ), 3.31 (s, 2H, $-SO_2NH_2$).

3-[N-(1-But-2-enyl)amino]-4-phenoxy-5-sulfamylbenzoic Acid (7). The procedure of Feit, et al.[14] was used for the alkylation of 6. Compound 6 (400 mg, 1.3 mmol) and 400 µl of crotyl bromide were dissolved and refluxed in absolute ethanol for 4 h. TLC showed partial conversion to the N-alkylated ethyl ester. An additional 400 µl of crotyl bromide was added and the reaction stirred under reflux overnight. The solution was evaporated to dryness and the residue column chromatographed on silica gel 60 (CH_2Cl_2-EtOAc 95:5) to afford 180 mg of the N-alkylated ethyl ester. Saponification of this product was accomplished by heating in 3.0 ml of 1.0 N aqueous NaOH on a steam bath for 45 min. Upon cooling and acidification with conc. HCl, a white precipitate formed which was filtered and dried in vacuo to afford 132 mg (28% based upon 6) of product as a colorless solid; mp 238-240°C. ^{1}H-NMR ($(CD_3)_2CO$) (TMS) δ 7.95 (d, 1H ArH), 7.63 (d, 1H, ArH), 7.10 (m, 5H, -O-φ), 6.48 (s, 2H, $-SO_2NH_2$), 5.49 (m, 2H, -HC=CH-), 3.80 (m, 2H, $-CH_2NH-$), 1.61 (m, 3H, $-CH_3$); m/e 362.0935 ($C_{17}H_{18}N_1O_5S$ requires 362.0935).

[N-Butyl-2,3-$^2H_2$]-bumetanide(8). Compound 7 (36 mg, 0.099 mmol) and 24 mg of tris(triphenylphosphine)rhodium chloride were dissolved in 4.0 ml of benzene-ethanol (1:1) and stirred at room temperature for 16 h under 1.0 atm of deuterium gas. The solvents were removed in vacuo and the dark residue was dissolved in acetone and chromatographed on two 20 x 20 cm x 0.25 mm silica gel 60 plates (F-254) with CH_2Cl_2-MEOH (8:2) to afford 13.3 mg (37%) of colorless solid; mp 224-225°C (lit.[14] mp 230-231°C). ^{1}H-NMR and R_f (~ 0.55) were identical to that of authentic bumetanide except for spectral differences produced by the presence of deuterium. ^{1}H-NMR ($(CD_3)_2CO$) (TMS) δ 7.87 (d, 1H, ArH), 7.60 (d, 1H, ArH), 7.06 (m, 5H, -O-φ), 6.50 (s, 2H, $-SO_2NH_2$), 3.13 (m, 2H, $-CH_2NH-$), 1.25 (m, 4H, -CHDCHD-), 0.85 (t, 3H, $-CH_3$). Mass spectrometry indicates d_0 = 3.13%, d_1 = 11.67%, d_2 = 69.11%, d_3 = 12.31%, d_4 = 2.96% and d_5 = 0.82%.

[N-Butyl-2,3-$^3H_2$]-bumetanide (9). Compound 7 (22.0 mg, 0.06 mmol) and 15 mg of tris(triphenylphosphine)rhodium chloride were dissolved in 0.75 ml of benzene-ethanol (1:1) and stirred for 16 h under 5.0 Ci (0.086 mmol) of carrier free tritium gas. The reaction solution was evaporated to dryness in vacuo, 10 ml of

absolute ethanol added to the residue, the volatiles evaporated, and 10 ml more ethanol added and evaporated to exchange off labile tritium. The residue was chromatographed on two 20 x 20 cm x 0.25 mm silica gel plates against authentic bumetanide as a standard (CH_2Cl_2-MEOH 8:2) to afford 352 mCi of product (~ 99% radiochemical purity) which was dissolved in 100 ml of absolute ethanol and stored at 5°C. A 15 ml aliquot of this stock solution was evaporated *in vacuo* and dissolved in 50 ml of methanol and quantitated by UV spectroscopy (390 nm-215 nm) using unlabelled bumetanide as a reference standard. The specific activity obtained was 16 Ci/mmol (43 mCi/mg).

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