SYNTHESIS OF TRITIATED CROMOLYN DIETHYL ESTER

Steven D. Wyrick and V. Ranga[a]

Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27514

[a]Center for Environmental Health and Medical Sciences, Department of Medicine, School of Medicine, University of North Carolina Chapel Hill, NC 27514

Summary

We report here the synthesis of tritiated cromolyn diethyl ester of high specific activity and acceptable stability for use in in vitro and in vivo models of experimental asthma in the guinea pig. Preparation of an aryl brominated precursor, 1,3-bis-(2-acetyl-3-hydroxy -6-bromophenoxy)-2-propanol, followed by catalytic debromination with tritium gas and two subsequent synthetic steps afforded tritiated cromolyn diethyl ester with a specific activity of 135 mCi/mg (71 Ci/mmol).

Key Words: Cromolyn, 1,3-bis-(2-acetyl-3-hydroxy-6-bromophenoxy) -2-propanol, catalytic debromination, deuterium, tritium.

Introduction

Cromolyn is a structurally unique drug that inhibits, but does not reverse, antigen-induced bronchoconstriction.[1] Although the precise mechanism of action of cromolyn is unclear, it appears to block the release of chemical mediators of Type I hypersensitivity reactions from mast cells.[2] In addition, there is evidence that it inhibits vagal reflex pathways through its effects on irritant receptors[3]; these actions presumably explain its beneficial effect in the prevention of allergic as well as exercise-induced asthma.[4]

Due to the poor absorption of cromolyn across the gastrointestinal tract after oral ingestion, the drug is used topically in humans.[5] This observation raises a logical question as to the access of the drug to its principal target area, i.e., mast cells and irritant receptors, which are both situated in the subepithelial compartment in mammalian airways. Furthermore, the recent observation that lung fibroblasts and alveolar macrophages may also be a source of

0362-4803/84/010037-08$01.00

Received August 10, 1983
Revised September 16, 1983

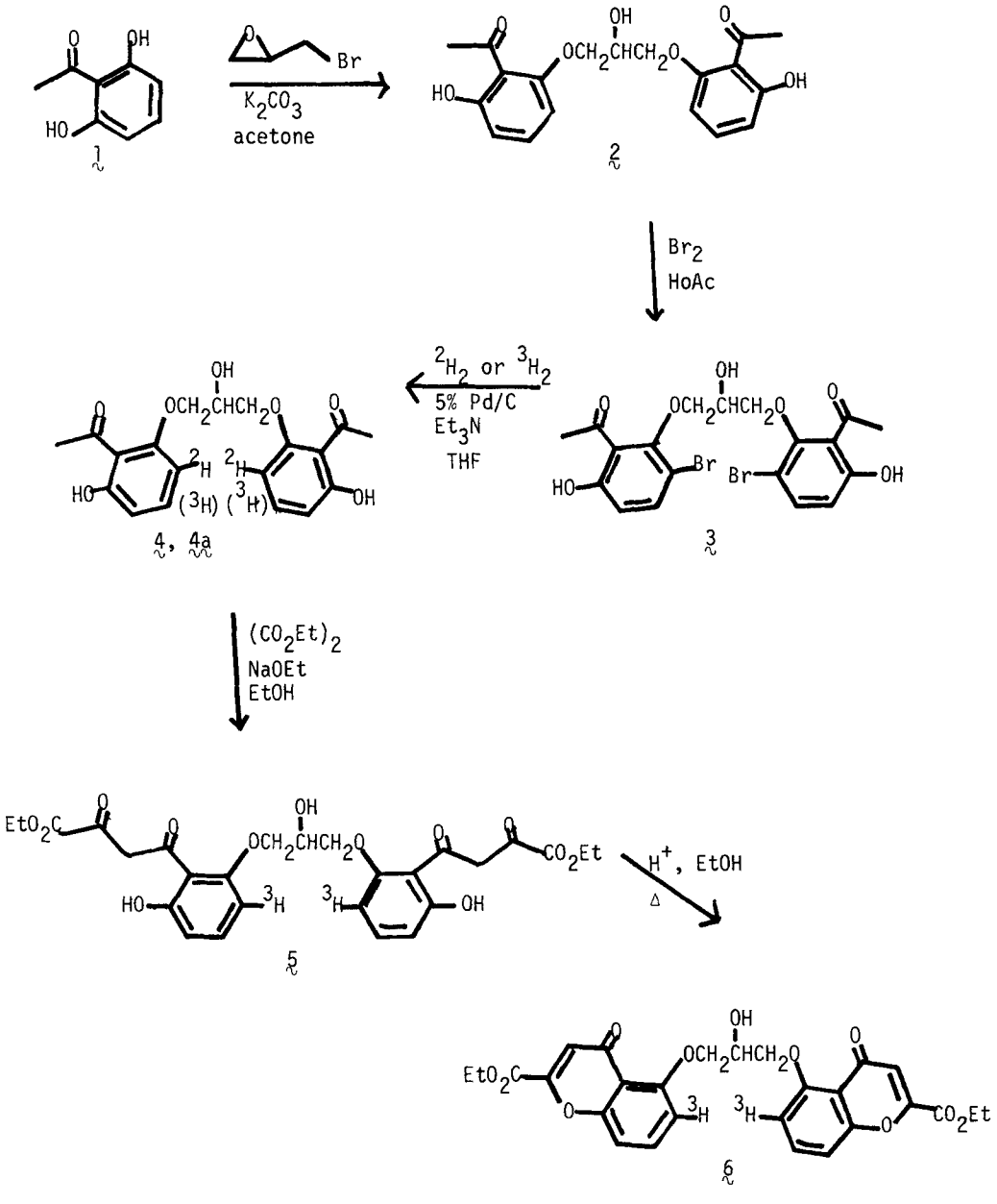
arachidonic acid metabolites including the prostaglandins and leukotrienes,[6-7] suggests another potential unexplored site of action by cromolyn. In order to investigate these issues, we report here, the successful synthesis of radiolabelled cromolyn of high specific activity (71 Ci/mmol) and acceptable stability for use in in vitro Ussing chambers for measurement of bioelectric properties and bidirectional transport of cromolyn across airway and gastrointestinal epithelia.[8] In addition, the technique of disaggregation and isolation of relatively pure populations of airway epithelial cells may permit studies of binding and uptake of ^{3}H-cromolyn[9] by the epithelial cells and alveolar macrophages utilizing autoradiographic and immunofluorescent techniques.

Nonspecifically labelled cromolyn[5] has been prepared at lower specific activity by catalytic exchange with tritium gas but is not available in the United States. The chemistry of unlabelled cromolyn has been discussed in detail elsewhere.[10]

Discussion

The synthetic scheme employed for tritiated cromolyn diethyl ester is shown in Figure 1. Upon bromination of 2 with bromine in acetic acid, a pure compound was obtained as a solid. ^{1}H-NMR indicated monobromination of both aromatic rings as evidenced by an AB quartet (see Experimental Section for spectral data). Since bromination at the 4 position would give the same m/e value and similar aromatic signals by ^{1}H-NMR as the 6-bromo product, additional structural verification was required to distinguish between the two possible isomers. Upon acetylation (Ac_2O/pyridine) of 3, the ^{1}H-NMR spectrum showed a 0.37 ppm downfield shift for the proton ortho to the acetylated phenolic hydroxyl with no shift change for the proton ortho to the bromine. Therefore, there must be a proton ortho to the phenolic hydroxy and the bromine must be in the 6 position since a proton in this position should not experience a shift change upon acetylation. In an attempt to introduce the label as near to the end of the synthesis as possible, 3 was treated with diethyl oxylate in ethanol in the

FIGURE 1


K2CO3
acetone
Br2
HoAc
2H2 or 3H2
5% Pd/C
Et3N
THF
(CO2Et)2
NaOEt
EtOH
H+, EtOH
Δ

presence of sodium ethoxide under conditions of a Claisen condensation to afford the dibromo derivative of 5. However, attempts to debrominate this compound under 1.0 atm of H_2 in the presence of 5% Pd/C and triethylamine resulted not only in debromination but also in reduction of the labile keto carbonyl functions located adjacent to the ester functions. Alternatively, 1,3-bis-(2-acetyl- 3-hydroxy-6-bromophenoxy)-2-propanol (3) underwent debromination smoothly under the same conditions with hydrogen and deuterium gas to afford 4a without reduction of the aryl carbonyl function. Some scrambling did occur as evidenced by mass spectral analysis of deuterium incorporation into 4a which is not unusual for reductions catalyzed by palladium. Actual deuterium incorporation predicted a specific activity upon tritiation of ~69 Ci/mmol. Reduction of 3 with tritium gas under identical conditions afforded 4 with a specific activity of 71 Ci/mmol which was converted to tritiated cromolyn diethyl ester (6) in two subsequent steps with a specific activity of 71 Ci/mmol (135 mCi/mg). Conversion of 1,3-bis-(-2-acetyl-3-hydroxy-[6-^{3}H]-phenoxy)-2-propanol (4) to the diester 5 with diethyl oxalate and sodium ethoxide in ethanol was accomplished by a modification of the procedure previously reported[11] for the nonlabelled compound in order to better facilitate a much smaller scale and handling of labelled product. It was found that a reaction time of 2.0 h was sufficient for complete conversion of 2 to the unlabelled derivative of the diester 5 as opposed to 20 h reported previously. This shorter reaction time is helpful in circumventing the extensive radiochemical decomposition which normally accompanies the heating of high specific activity compounds for extensive periods of time. Subsequently, 5 was refluxed 20 min in absolute ethanol in the presence of a catalytic amount of HCl to afford tritiated cromolyn diethyl ester (6) in 13% yield based upon 4. Attempts were made to convert the labelled diester 6 to the diacid, cromolyn, by the reported procedure[10] which involved heating the diester in boiling ethanol in the presence of aqueous 1.0 N NaOH. Employing this procedure for the high specific activity diester 6 and a lower

specific activity sample (from isotopic dilution) afforded only decomposition products. The pure product 6 was stored at 4°C in absolute ethanol solution at a concentration of 0.316 mCi/ml and showed no detectable decomposition after 4 weeks by TLC-radioscan.

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 $CDCl_3$ or $(CD_3)CO$ (TMS) as solvent. Gas-liquid chromatography was performed using a Shimadzu GC-8A (FID) chromatograph (2.0 m glass column, 3% OV-17 on chromasorb, 30 ml/min). Radio-purity was determined using an Autochron LB-2722 radioscanner. 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 analysis. Elemental compositions of novel compounds were determined by high resolution mass spectrometry using an AEI MS-902 mass spectrometer.

1,3-Bis(2-acetyl-3-hydroxyphenoxy)-2-propanol (2). This compound was prepared from 2,6-dihydroxyacetophenone and epibromohydrin according to the procedure previously reported.[11] The yield after chromatography (silica gel 60, CH_2Cl_2, CH_2Cl_2-ethyl acetate 9:1) was 20% of a light yellow solid; mp = 166 - 168°C (lit.[11] 165 - 166°C). ^{1}H-NMR (δ) $(CD_3)_2CO$ (TMS) 7.58 - 6.48 (m, 6H, ArH_6), 4.44 (m, 5H, $-OCH_2CHOHCH_2O-$), 2.77 (s, 6H, CH_3CO).

1,3-Bis(2-acetyl-3-hydroxy-6-bromophenoxy)-2-propanol (3). To a suspension of 100 mg (0.278 mmol) of powdered 2 in 3 ml of glacial acetic acid was added dropwise 89 mg (0.555 mmol) of bromine in 1.0 ml of glacial acetic acid. During the addition, a solution was obtained followed by precipitation of the product while stirring 20 min at room temperature. The precipitate was filtered, washed with ether and dried to afford 274 mg (62%) of product as a light yellow solid, mp =

168 - 170°C. ^{1}H-NMR (δ) $(CD_3)_2CO$ (TMS) 7.69 (d, 2H, ArH_2), 6.67 (d, 2H, ArH_2), 4.44 (m, 5H, $-OCH_2CHOHCH_2O-$), 2.79 (s, 6H CH_3CO); m/e 515.9422 ($C_{19}H_{18}Br_2O_7$ requires 515.9422).

1,3-Bis(2-acetyl-3-hydroxy-[6-^{2}H]-phenoxy)-2-propanol (4). A solution of 70 mg (0.135 mmol) of 3 and 200 µl of triethylamine in 3.0 ml of dry THF was stirred for 3 h at room temperature under 1.0 atm of deuterium gas in the presence of 15 mg of 5% Pd/C. The reaction suspension was filtered through a small Celite column and the volatiles removed under a stream of N_2. The residue was partitioned between CH_2Cl_2-H_2O and the organic layer dried (Na_2SO_4) and evaporated in vacuo to afford 43 mg (88%) of pure deuterated product; mp = 163 - 166°C. ^{1}H-NMR (δ) $(CD_3)_2CO$ (TMS) 7.47 (d, 2H, ArH_2), 6.62 (d, 2H, ArH_2), 4.42 (m, 5H, $-OCH_2CHOHCH_2O-$), 2.76 (s, 6H, CH_3CO); mass spectrometry indicates d_0 = 1.72%, d_1 = 9.89%, d_2 = 48.07%, d_3 = 29.74%, d_4 = 9.23%, d_5 = 1.35%.

Aryl Tritiated Cromolyn Diethyl Ester (6). A solution of 19.4 mg (0.0375 mmol) of 3 and 100 µl of triethylamine in 0.5 ml of dry THF was stirred for 3 h at room temperature under 5.0 Ci (0.086 mmol) of carrier free tritium gas in the presence of 10 mg of 5% Pd/C. The reaction was filtered through a Celite-Na_2SO_4 pipet column to remove the catalyst and the filtrate evaporated in vacuo. The residue was dissolved immediately in 6.0 ml of CH_2Cl_2, extracted twice with H_2O, dried (Na_2SO_4) and the volume adjusted to 10 ml with CH_2Cl_2. A 100 µl aliquot was silated for 2 h with Regisil ^{R} and quantitated by gas-liquid chromatography using 1,3-bis(p-methoxyphenoxy)-2-propanone as an internal standard and also diluted and subjected to liquid scintillation counting to afford a specific activity of 71 Ci/mmol. The total yield of 4 was 1.02 Ci and was found to be > 95% radiochemically pure by TLC-radioscan (SiO_2:CH_2Cl_2-EtOAc 9:1). The CH_2Cl_2 was evaporated in vacuo and the residue (~1.01 Ci, 0.014 mmol of 4) was dissolved in 2.0 ml of benzene and 1.0 ml of absolute ethanol. This solution was treated with 10 mg (0.07 mmol) of diethyl oxalate and 0.091 mmol of 1.16 M ethanolic sodium ethoxide and refluxed for 2 h. The solvents were evaporated in

vacuo and the residue swirled with H_2O. The aqueous suspension was extracted with ether, acidified with 1.0 N HCl and the cloudy suspension extracted with CH_2Cl_2. The organic extracts were dried (Na_2SO_4) and evaporated in vacuo to afford crude 5 which was dissolved and refluxed for 20 min in 3.0 ml of absolute ethanol and 1 drop of 1.0 N HCl. TLC-radioscan indicated ~75% purity of crude 6. The volatiles were removed in vacuo and the residue taken up in 500 ml of absolute ethanol. After attempts were made to saponify this diester, 125 ml of the above solution was concentrated and purified by preparative TLC using a 20 cm X 20 cm X 0.25 mm silica gel 60 (F-254) plate with authentic cromolyn diethyl ester as a reference standard (CH_2Cl_2-methanol 95:5, R_f = ~0.65) to afford 31.6 mCi (13% based upon 4) of > 95% pure cromolyn diethyl ester (6). The product was stored at 4°C in absolute ethanol at a concentration of 0.316 mCi/ml.

Acknowledgements

We wish to thank Mr. Chris Wyrick and Mr. George Taylor of the Research Triangle Institute, Research Triangle Park, N.C., 27709 for technical assistance with the tritium gas reduction. This work was supported by grants HL29222-01 from the National Institutes of Health and CR 803792-02 from the U.S. Environmental Protection Agency.

References

1. Assem E.S.K. and Mongar J.L. - Int. Archs. Allergy 38: 68 (1970).
2. Cox J.S.G. - Br. J. Dis. Chest 65: 189 (1971).
3. Dixon M., Jackson D.M., Richards I.M. - Br. J. Pharmacol. 80: 11 (1980).
4. Davies S.E. - Br. Med. J. 3: 593 (1968).
5. Moss G.F., Jones K.M., Ritchie J.T. and Cox J.S.G. - Toxicol. Appl. Pharmacol. 17: 691 (1970).
6. Ozaki T., Rennard S. and Crystal R.G. - Am. Rev. Resp. Dis. 127: 275 (1983) (abstract).

7. Elias J.A., Zurier R.B., Rossman M.D. and Daniele R.P. - Am. Rev. Resp. Dis. 127: 61 (1983) (abstract).

8. Gatzy J.T. - Fed. Proc. 38: 2234 (1979).

9. Mazurak N., Berger G. and Pecht I. - Nature 286: 722 (1980).

10. Cox J.S.G., Beach J.E., Blair A.M.J.N., Clarke A.J., King J., Lee T.B., Loveday D.E.E., Moss G.F., Orr T.S.C., Archie J.T. and Sheard P.- Advances in Drug Research, 5: 115 (1970).

11. Fisons Pharmaceuticals Ltd., British Patent Appl., March 25, Dec. 9 and 17 (1965). - Chem. Abst. 67: 100002d (1967).