SYNTHESIS OF 2,5-DIHYDROXY[CARBOXY-^{14}C]BENZOIC ACID FOR ELECTROCHEMICAL PROTEIN MODIFICATION

Varghese John, Jonathan L. Vennerstrom, and Thomas J. Holmes, Jr.

Department of Medicinal Chemistry
College of Pharmacy
University of Minnesota
Minneapolis, Minnesota 55455

SUMMARY

The synthesis of 2,5-dihydroxy[carboxy-^{14}C]benzoic acid (gentisic acid) is described. The procedure utilized [carboxy-^{14}C]salicylic acid as a starting material. Salicylic acid was converted to gentisic acid using potassium persulfate hydroxylation. The mixture of hydroxylation products in this reaction was easily separated by UV-visualized quartz column chromatography to obtain the desired isomer in high purity (overall yield of 38%). This procedure may be used for micro and semimicro preparation of the title compound.

Key Words: Carbon-14, gentisic acid, quartz column, hydroxylation.

INTRODUCTION

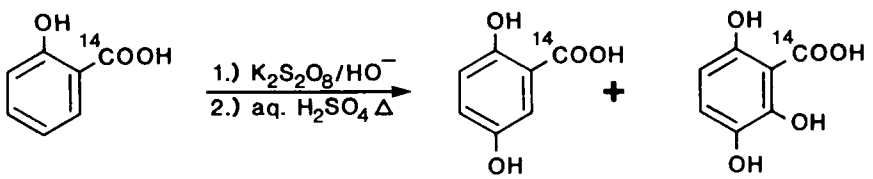
Salicylic acid (1), a primary metabolite of aspirin, has been shown to be equipotent to aspirin in the reduction of prostaglandin formation and inflammatory exudates *in vivo*, but is a weak inhibitor of PGH-synthase *in vitro* (1). It has been suggested that these *in vivo* antiinflammatory effects might be due to formation of an "active metabolite," gentisic acid (2; ref. 2). This has has been confirmed by the observed *in vitro* activity of gentisic acid as an inhibitor of prostaglandin synthesis (3). Further oxidation of gentisic acid to a reactive quinone species could lead to irreversible inhibition of cyclooxygenase by covalent modification. This hypothesis has been supported by

0362-4803/86/030313-04$05.00

Received August 28, 1985
October 8, 1985

the observation that gentisic acid caused a time-dependent irreversible inhibition of PGH-synthase when subjected to oxidative electrolysis in the presence of the enzyme (4).

In order to evaluate the quantitative aspects of this inhibitory phenomenon, the synthesis of ^{14}C-labeled gentisic acid was required. The synthesis of [carboxy-^{14}C]gentisic acid had been previously reported utilizing $^{14}CO_2$ (5) but this procedure is inconvenient for the synthesis of small quantities. We report herein a straightforward method for synthesis of radiolabeled gentisic acid starting from the available [carboxy-^{14}C]salicylic acid utilizing a modified hydroxylation procedure (6).



1 2 3

EXPERIMENTAL

Materials: [Carboxy-^{14}C]salicylic acid (53.8 mCi/mmol) was obtained from New England Nuclear Corporation, Boston, Massachusetts. Reagent grade potassium persulfate was obtained from J. T. Baker Chemical Company, Glen Ellyn, Illinois. Zinc silicate powder was obtained from Dupont Luminescent Chemicals Division many years ago. Merck silica gel, grade 60 (230-400 mesh) was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. A quartz tube (0.7 x 30 cm) was obtained from the University of Minnesota Glass Technology Services. Ultraviolet detection of chromatographic separation utilized a hand-held Mineralight Model UVGL-58 with short (254 nm) and long (366 nm) wavelength capability. Radioactivity counting was performed in Aquasol-2 (New England Nuclear) with a Beckman LS6800 scintillation counter with a counting efficiency of 72 percent. The purity of reagents and products was determined by thin-layer chromatography on silica gel plates (Analtech No. 47521 hard-layer) utilizing methanol/chloroform 1:9 v/v as the eluent. ^1H-NMR

determinations were made with a JEOL FX-90Q (90 MHz) spectrometer in acetone-d_6 (Chemical Dynamics Corporation, South Plainfield, New Jersey). All other solvents and reagents were commercially available reagent grade materials.

2,5-dihydroxy[carboxy-^{14}C]benzoic acid: To 0.1 mg (0.7 μmol) of [carboxy-^{14}C]salicylic acid (53.8 mCi/mmol) mixed with 13.7 mg of unlabelled salicylic acid in 2.0 ml of aqueous 0.23 N potassium hydroxide at 0°C (ice bath) was added slowly dropwise 32 mg (0.12 mmol) of potassium persulfate dissolved in 2.0 ml of water. After the addition was complete, the ice bath was removed and the reaction mixture stirred for 15 hr at room temperature. Following this period the mixture was acidified by the addition of 0.7 ml concentrated sulfuric acid and then boiled for 45 min. The resulting solution was extracted with ethyl acetate (30 ml). After drying over anhydrous sodium sulfate this organic solution was evaporated *in vacuo* to provide 12 mg of a brownish solid, which was a mixture of salicylic acid, gentisic acid, and 2,3,5-trihydroxybenzoic acid (3).

This mixture was dissolved in 0.5 ml of 5% methanol/chloroform (v/v) and applied to the top of a wet-packed short column of silica gel (1.0 gm; Merck 230-400 mesh), that had been intimately mixed with 15 mg of zinc silicate as a photosensitizer, in a quartz glass tube (0.7 x 30 cm). This column was then eluted with 25 ml of 5% methanol/chloroform, followed by 10% methanol/chloroform as necessary. The progress of the separation was followed by intermittent visualization of bands using a hand-held ultraviolet lamp (Mineralight) as generally suggested by others (7). The fastest running salicylic acid band appeared green under short-wavelength (254 nm) light; the desired gentisic acid band that followed appeared blue fluorescent under long-wavelength (366 nm) ultraviolet light. Collection of the gentisic acid band provided 6 mg (38% yield) of [carboxy-^{14}C]gentisic acid (400 μCi/mmol) after evaporation. The slower running trihydroxybenzoic acid was not quantitated in this experiment. [Carboxy-^{14}C]gentisic acid prepared in this manner was identical to a gentisic acid reference sample by TLC (R_f = 0.16;

silica gel/methanol:chloroform 1:9) and ^{1}H-NMR (acetone-d_6: δ8.4 (bs, 1H), 7.4 (d, J_{AX} = 1.8 Hz, 1H), 7.2 (dd, J_{AB} = 9.0 Hz, J_{AX} = 1.8 Hz; 1H), 7.0 (d, J_{AB} = 9.0 Hz).

DISCUSSION

This described procedure enables the rapid and convenient preparation of [carboxy-^{14}C]gentisic acid, a radiolabelled aspirin metabolite, and potentially other hydroxylated salicylate derivatives as well. In principal, this procedure could be optimized for the preparation and purification of other di- and tri-hydroxybenzoic acid derivatives according to previously observed chemical selectivity in this hydroxylation procedure (6). The quantitative attachment of this quinonoid precursor to enzymes and proteins other than cyclooxygenase (4) via this electrochemical technique is under investigation to prepare radiolabelled macromolecules and specifically functionalized (electromodified) proteins.

REFERENCES

1. Rainsford K.D. - Aspirin and the Salicylates, pp. 80-81, Butterworths, London (1984)
2. Blackwell G.J., Flower R.J. and Vane J.R. - Biochim. Biophys. Acta 398: 178-190 (1975)
3. Flower R.J. - Pharm. Rev. 26: 33-67 (1974)
4. Holmes T.J., Jr., Vennerstrom J.L. and John V. - J. Biol. Chem. in press (1985)
5. Medenwald H. and Haberland G.L. - Z. Physiol. Chem. 306: 229-234 (1957)
6. Schock R.V., Jr. and Tabern D.L. - J. Org. Chem. 16: 1772-1775 (1951)
7. Chiong H-C. and Wang P-L. - J. Chem. Ed. 60: 419 (1983)