SYNTHESIS OF [γ-^{14}C]-γ-OXO-2-DIBENZOFURANBUTANOIC ACID (FUROBUFEN) AND 2-DIBENZOFURANACETIC[CARBOXY-^{14}C] ACID

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SUMMARY

The syntheses of [γ-^{14}C]-γ-oxo-2-dibenzofuranbutanoic acid (furobufen) 7 and 2-dibenzofuranacetic[carboxy-^{14}C] acid 3b, a major metabolite of furobufen, are described. A by-product, the dialkylated malonate 6 obtained in the synthesis of 7, was isolated and characterized.

Key words: γ-oxobutanoic acid, acetic acid, ^{14}C, furobufen

INTRODUCTION

γ-Oxo-2-dibenzofuranbutanoic acid (furobufen) has the pharmacological profile of an anti-inflammatory agent (1). Completion of the studies of the metabolic disposition of the compound in laboratory animals and man required [^{14}C]furobufen. Previous studies (2) indicated that the γ-carbon on the butanoic acid chain was metabolically stable and a synthesis of the compound labelled in that position was developed. The syntheses of [^{14}C]furobufen and 2-dibenzofuranacetic[carboxy-^{14}C] acid, a major metabolite of furobufen, are described herein.

DISCUSSION

γ-Oxo-2-dibenzofuranbutanoic acid 7 has been previously prepared by a Friedel-Crafts reaction of dibenzofuran with succinic anhydride (3). However, since only the γ-carbon was to be labelled, the oxobutanoic acid chain had to

be built up stepwise. This was achieved by the reaction sequences shown in Scheme 1. Dibenzofuran 1 was acylated with [1-^{14}C]acetyl chloride and the resulting methyl ketone was a common intermediate in the synthesis of both the oxobutanoic acid 7 and the substituted acetic acid 3b.

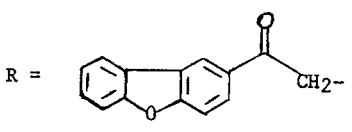
Application of the Willgerodt reaction to 2 followed by basic hydrolysis of the resulting amide 3a, as first described by Gilman and Avakian (4), gave the acetic acid derivative 3b in a good yield. Since the Willgerodt reaction involves the rearrangement of the carbonyl carbon from an internal position to the end of the carbon chain (5), the [^{14}C]label of the carboxylic acid 3b is in position 1 of the acetic acid moeity. Thus, in this three-step sequence 2-dibenzofuranacetic[carboxy-^{14}C] acid 3b, specific activity 14.6 μCi/mmole, was obtained in a 15% overall yield from a microscale run.

Scheme 1

The γ-oxobutanoic acid chain was synthesized from the methyl ketone 2 in the following manner. Bromination of the methyl group with pyridinium bromide perbromide gave the monobromoketone 4a in 90% yield. The dibromoketone 4b was obtained as a minor by-product. Displacement of the bromine atom of 4a with sodium diethylmalonate in N,N-dimethylformamide gave the keto diester 5a in 51% yield after purification by column chromatography. The formation of a second product, the substituted malonic ester 6, reduced the yield of the desired keto diester 5a. Presumably an equilibrium established between the diester 5a, diethylmalonate and their respective anions 10 and 8, favors the anion 10. The concentration of the anion 10 will therefore increase with the formation of 5a

$$\ominus CH(CO_2Et)_2 + RCH(CO_2Et)_2 \rightleftharpoons CH_2(CO_2Et)_2 + \overset{\ominus}{R}C(CO_2Et)_2$$

8 5a 9 10

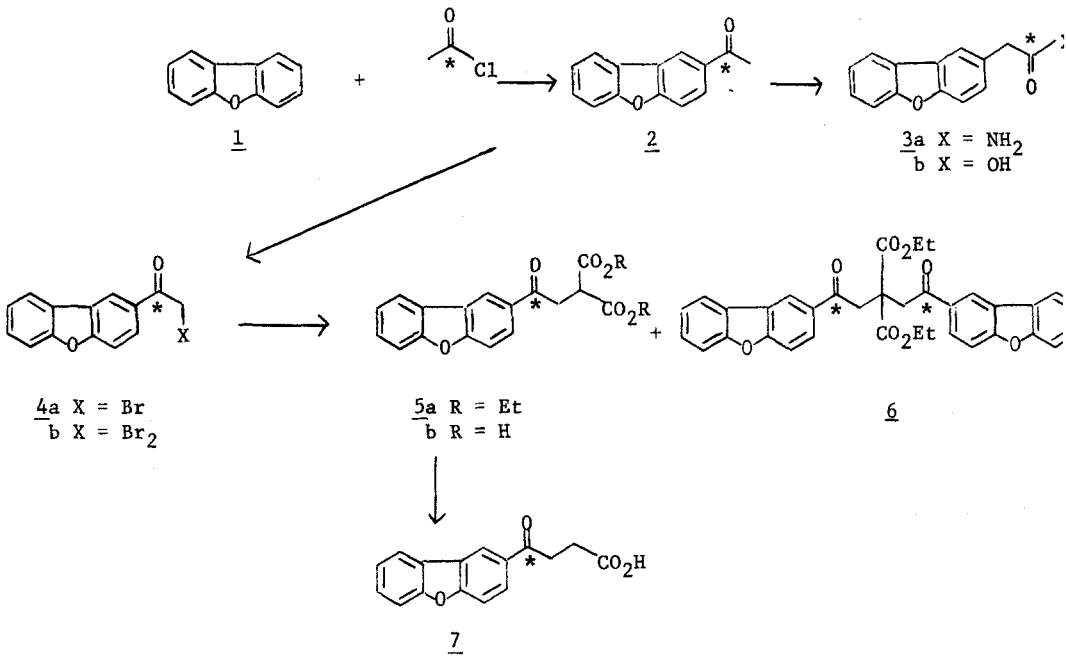


4a

6

and also react with the bromoketone 4a to form the disubstituted malonic ester 6.

Scheme 1



The malonic ester 6 was characterized by i.r., n.m.r. and mass spectral analysis. The comparison of n.m.r. and mass spectra of the keto-diester 5a with those obtained for 6 was particularly informative. The n.m.r. spectrum of 6 was devoid of a malonate proton signal [C<u>H</u>$(CO_2Et)_2$] and contained a singlet at signal δ4.25 for the α-keto protons. The mass spectra of both 5a and 6 (M^+ 368 and 576 respectively) were consistent for acyldibenzofuran derivatives.

The synthesis of the γ-oxobutanoic side chain and [^{14}C]furobufen 7 was completed by hydrolysis of the diester 5a, followed by decarboxylation of the corresponding diacid 5b. The hydrolysis was efficiently accomplished in aqueous methanolic potassium hydroxide. The diacid 5b was unusually stable

to mineral acid and decarboxylation occurred only after prolonged refluxing in acetic acid containing a trace of sulfuric acid. The final product, [^{14}C]furobufen 7 (5.2 mCi, specific activity 3.7 mCi/mmole), purified by recrystallization from ethyl acetate-petroleum ether (30-60°), was obtained in 17% overall yield from [1-^{14}C]acetyl chloride.

EXPERIMENTAL

The infrared spectra were recorded on a Perkin-Elmer diffraction grating instrument model 237, the n.m.r. spectra on a Varian A60A instrument, and the mass spectra on a LKB 9000 spectrometer, respectively. The melting points were taken on a Thomas Hoover apparatus and are uncorrected. EM Laboratories 0.25 mm precoated Silica Gel G F-254 plates were used for thin layer chromatography and Merck silica gel 60 (70-230 mesh) was employed for column chromatography. Kodak RP/R14 X-Omat medical X-ray films were used in the autoradiography. The radioactivity was measured with a Packard Tri-Carb Model 3375 liquid scintillation spectrometer. [1-^{14}C]Acetyl chloride, 30 mCi (specific activity 3.88 mCi/mmole) and 8 mCi (specific activity 55 mCi/mmole) were purchased from New England Nuclear Corp., Boston, Mass.

1-(2-Dibenzofuranyl) [1-^{14}C]ethanone (2). A 50 ml three-necked reaction flask fitted with a magnetic stirrer, a dropping funnel containing 5 ml methylene chloride and a stopper, was connected to a vacuum line, and the system was evacuated (0.3 mm Hg). A breakseal vial containing [1-^{14}C]acetyl chloride (7.73 mmole, 3.88 mCi/mmole) was connected to the vacuum line, evacuated, and after breaking the seal, the acetyl chloride was frozen out by cooling the reaction flask with liquid nitrogen. After all the reagent had been transferred, the vacuum was released with dry nitrogen gas and methylene chloride was added; the liquid nitrogen coolant was removed and replaced with an ice-water bath. Dibenzofuran (1) (1.69 g, 10.1 mmole) dissolved in 5 ml methylene chloride, was added and the dropping funnel was washed with an additional 5 ml of solvent. Aluminium chloride (3.0 g) was then added in one portion to the stirred solution. The resulting thick suspensi n was stirred at 0° for 15 min and at room temperature for 4 hr.

The reaction mixture was poured onto crushed ice containing 10% hydrochloric acid and the flask was well washed with chloroform and water.
The aqueous fraction was separated and extracted with chloroform. The combined organic phase was washed with water and saturated saline solution, dried over anhydrous magnesium sulfate ($MgSO_4$) and concentrated. The resulting product (1.79 g) was chromatographed on a column of silica gel (90 g) with benzene as eluent. The title compound 2 (1.27 g) was obtained as an off-white solid in 77% yield: i.r. ($CHCl_3$) 1670 cm^{-1} (C=O).

In the acylation reaction carried out in the same manner on a microscale using [1-^{14}C]acetyl chloride (11.3 mg, 0.142 mmole), dibenzofuran (29.2 mg, 0.174 mmoles) and 52 mg $AlCl_3$, the labelled methyl ketone 2 (9 mg) was obtained in 33% yield.

2-Dibenzofuranacetic[carboxy-^{14}C] acid (3b)

Willgerodt Reaction. The methyl ketone 2 (9 mg) was transferred with ether to a pyrex tube (6 mm x 100 mm), the solvent was evaporated and the tube was charged with 0.3 ml dioxane, 33 mg sulfur, 0.33 ml ammonium sulfide and a micro stirring bar. After freezing its contents with dry ice, the tube was sealed, placed in an oil bath at 160°, and the reaction mixture was heated at this temperature with stirring for 15 hr. The tube was then cooled to -78°, opened and the mixture taken up in water and chloroform. The aqueous fraction was extracted several times with chloroform the combined chloroform extracts were then washed with water, saturated saline solution, dried ($MgSO_4$) and concentrated. The resulting amide was hydrolyzed to the acid without prior purification.

Hydrolysis. The crude amide 3a was refluxed for 3 hr in 20 ml 15% potassium hydroxide in aqueous ethanol (1:1). The reaction mixture was cooled, diluted with water and extracted with ether to remove all neutral material. The aqueous fraction was cooled with crushed ice, acidified with concentrated hydrochloric acid and extracted with ether. The combined ether extracts were washed with water, saturated saline solution, dried ($MgSO_4$) and concentrated.
The residue was diluted with 7 mg of unlabelled 2-dibenzofuranacetic acid and recrystallized from benzene to constant specific activity. The title compound, specific activity 14.6 ± 0.3 mCi/mmole, was obtained in 15% overall

yield.

The radiochemical purity of the acetic acid 3b was established in the following manner. A solution of 3b was spotted on three 5 x 20 cm TLC plates and these were developed in three solvent systems: 1) dioxane-hexane 60:40; 2) cyclohexane-acetone-acetic acid 50:40;1; 3) benzene-ethanol-acetic acid 80: 12:5. After drying, the plates were exposed to X-ray film for 15 hrs. The radioactive zones on the plates were located from the developed films and the silica gel was scraped from the plate and its radioactive content was measured by liquid scintillation spectroscopy (7).

2-Bromo-1-(2-dibenzofuranyl) [1-^{14}C]ethanone (4a). The ketone 2 (1.27 g, 6.05 mmoles) dissolved in 15 ml chloroform, was cooled in an ice-water bath and pyridinium bromide perbromide (1.91 g) was added portion-wise to the stirred solution. After stirring the reaction mixture for 15 min at 0°, the ice-bath was removed and the solution was stirred until all the brominating agent has dissolved (ca. 15 min). The solution was then poured onto ice-water and extracted with chloroform. The chloroform extract was washed with water, saturated saline solution, and dried ($MgSO_4$). After concentration, the crude product (2.17 g) was chromatographed on a column of silica gel (100 g) with hexane-benzene (2:8) as eluent. Three compounds were isolated: the first (135 mg) was the dibromoketone 4b: i.r.($CHCl_3$) 1725, 1680 cm^{-1}; n.m.r.($CDCl_3$)δ6.90(s, 1H, $C\underline{H}Br_2$), 7.3-8.8(m, 7H, aromatic). The second compound was the desired bromoketone 4a (1.61 g) obtained in 92% yield; mp 52 -3°; i.r. ($CHCl_3$) 1670 cm^{-1}; n.m.r.($CDCl_3$) δ4.53(s, 2H, $C\underline{H}_2Br$), 7.2-8.3(m, 7H, aromatic). Anal. Calc'd. for $C_{14}H_9BrO_2$: C, 58.15; H, 3.11. Found: C, 58.18; H,3.09. The third compound was the methyl ketone 2.

[γ-^{14}C]-α-Carboxy-γ-oxo-2-dibenzofuranbutanoic acid diethyl ester (5a).

Diethyl malonate (1.06 g, 6.63 mmole) dissolved in 5 ml anhydrous N,N-dimethylformamide (DMF) was added to a suspension of sodium hydride (290 mg of a 55% oil dispersion) in 10 ml DMF. The mixture was stirred for 30 min at room temperature in an atmosphere of dry nitrogen. The bromoketone 4a (1.61 g, 5.57 mmole), dissolved in 15 ml anhydrous DMF, was added dropwise and the resulting solution was stirred at room temperature for 2 hr. The reaction mixture was

then diluted with water and extracted several times with ethyl acetate. The combined extracts were washed with water and saturated saline solution, dried ($MgSO_4$) and concentrated. The resulting oil was chromatographed on a column of silica gel (185 g) with ethyl acetate-hexane 9:1 as eluent. Two compounds were isolated: The desired diester 5a, obtained as a crystalline solid (1.05 g) in 51% yield: i.r. ($CHCl_3$) 1740, 1720 (C = O, ester), 1675 (C = O, ketone) cm^{-1}; n.m.r. ($CDCl_3$)δ1.28 (t, J = 7Hz, 3H, CH_2CH_3, 3.8(m, 2H, CH_2CO), 4.2 (m, 1H, $CH(CO_2Et)_2$), 4.30(q, J = 7Hz, 2H, CH_2CH_3), 8.7-7.3(m, 7H, aromatic). The second compound (450 mg) was the dialkylated malonic ester 6: i.r.($CHCl_3$) 1725, 1675 cm^{-1}; n.m.r.($CDCl_3$)δ1.27(t, J = 7Hz, 6H, CH_2CH_3), 4.0-4.6(m, 8H, CH_2CH_3, $COCH_2$), 7.2-8.8(m, 7H, aromatic); m.s. $^m/e$ 576 (M^+). Anal. Calc'd. for $C_{35}H_{28}O_8$: C, 72.92; H, 4.86. Found: C, 72.79; H, 4.93.

[γ-^{14}C]-α-Carboxy-γ-oxo-2-Dibenzofuranbutanoic acid (5b). Potassium hydroxide (200 mg) dissolved in 0.5 ml water was added to a solution of the diester 3a (1.05 g) in 25 ml methanol. The reaction mixture was stirred at room temperature for 15 hrs, then concentrated, diluted with water and extracted with ether to remove all neutral material. The aqueous fraction was acidified with 10% hydrochloric acid and extracted with ethyl acetate. The extract was washed with water and saturated saline solution, dried ($MgSO_4$) and concentrated. The diacid 3b was obtained as a crystalline solid (0.91 g) in 100% yield: n.m.r. (d-DMSO) δ3.6-4.2 (m, 3H, $COCH_2CH$), 7.5-10.8(m, 9H, aromatic and CO_2H).

[γ-^{14}C]-γ-Oxo-γ-2-dibenzofuranbutanoic acid (furobufen) (7).

The diacid 8b (0.91 g, 2.9 mmole) was dissolved in 40 ml glacial acetic acid containing 2 drops of concentrated sulfuric acid and refluxed for 15 hr. The reaction mixture was concentrated, diluted with water and extracted with ethyl acetate. The extract was washed with water, saturated saline solution, dried ($MgSO_4$) and concentrated. The crude product (700 mg) was purified as follows: The orange coloured solid was placed on a column of silica gel (100 g) containing a layer of charcoal (3 g) 2 cm below the top of the gel. The column was eluted with dioxane-chloroform-hexane (40:30:30). While the colour had been removed, the radiochemical purity of the isolated product was unsatisfactory. Another chromatography on silica gel (100 g) using gradient

elution with benzene to benzene-ethyl acetate (1:1) and finally tetrahydrofuran gave a 98.9% pure compound. The radiochemical purity was improved to 99.7% by recrystallization from ethyl acetate-petroleum ether (30-60°). A mixed melting point of the [^{14}C]furobufen, obtained in 17% overall yield, with unlabelled furobufen showed no depression. The radiochemical purity of 7 was determined by TLC-autoradiography, as described for the acetic acid 3b, in three solvent systems: 1) methanol-chloroform 20:80; 2) hexane-dioxane 40:60; 3) methanol-chloroform 7:93.

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