SYNTHESIS OF [1-^{14}C-CARBOXY]-4-BENZOYLBENZOIC ACID

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SUMMARY

[1-^{14}C-Carboxy]-4-benzoylbenzoic acid has been prepared via a Grignard reaction with $^{14}CO_2$. 4-Bromobenzophenone was converted to (4-bromophenyl)-phenyldichloromethane using phosphorus pentachloride. The dichloro compound was reacted with methoxide ion to form 4-bromobenzophenone dimethylketal which was subsequently converted to its Grignard reagent, carboxylated with $^{14}CO_2$, and acid hydrolyzed to the final product. Using excess Grignard reagent, virtually quantitative conversion of $^{14}CO_2$ to product was obtained.

Key Words: [1-^{14}C-Carboxy]-4-benzoylbenzoic acid, photoaffinity probe, Grignard reaction, $Ba^{14}CO_3$, 4-bromobenzophenone dimethylketal.

The use of photoaffinity labeling reagents for the covalent labeling and characterization of biological binding sites has become a standard strategy in biochemical studies (1,2). A large number of useful reagents, normally precursors of carbenes or nitrenes, have been synthesized and used in a variety of systems. Galardy et al. (3) first recognized the potential usefulness of aromatic ketones, particularly benzophenone derivatives, as photochemically active labeling reagents. The photoactivation of such aromatic ketones produces a triplet diradical intermediate that is essentially inert to water, does not rearrange, and reacts preferentially with C-H bonds. Because of these advantages a number of photolabeling studies in biological systems have utilized benzophenone derivatives (4-8). One of the most recent and potentially useful derivatives is the ATP analogue, 3'-O-(4-benzoyl)-benzoyladenosine-5'-triphosphate, first prepared and used by Williams and Coleman (9) to label the mitochondrial F_1 ATPase. It has subsequently been used by other workers to label the chloroplast CF_1 ATPase (10) and the

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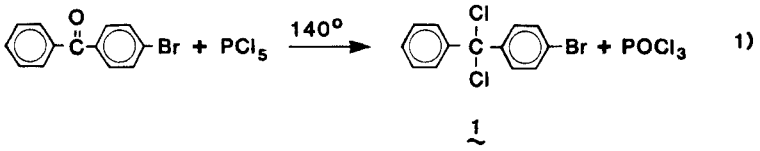
sarcoplasmic reticulum Ca^{2+} ATPase (11). Our own laboratory has shown that this analogue is an effective and specific photolabel of amino acid residues at or near the active site of myosin (12, 13). To identify these modified amino acids it became essential to prepare radiolabeled 4-benzoylbenzoic acid.

The synthesis of [1-^{14}C-carboxy]-4-benzoylbenzoic acid is reported here utilizing the reaction of the appropriate Grignard reagent and $^{14}CO_2$. The utilization of a three-fold excess of Grignard reagent over $^{14}CO_2$ in essentially the final step of the synthesis gave a nearly quantitative yield of product (based on CO_2) with the same specific radioactivity as the $Ba^{14}CO_3$ starting material.

The synthetic scheme was:



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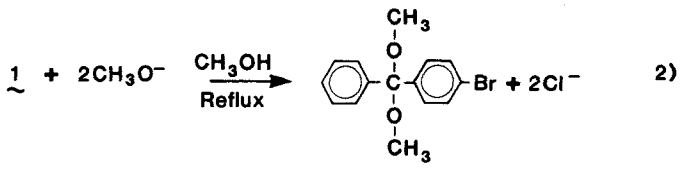

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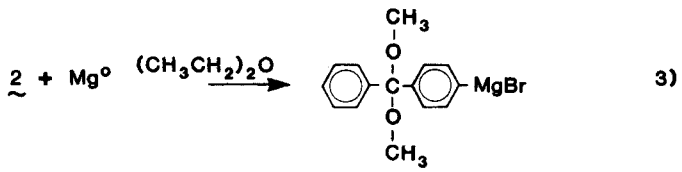

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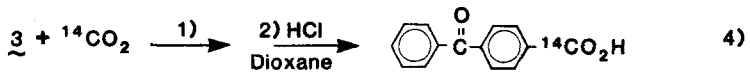

4

The carbonyl group of the benzophenone must be protected in order to prepare the Grignard reagent but it is not readily converted directly to a ketal. Consequently, the carbonyl group was first converted to a dichloride (1) which was subsequently converted to the ketal (2) by nucleophilic displacement of the chlorides by methoxide ion. The preparation of the Grignard reagent (reaction 3) was facile using the bromo compound (2), but it could not be prepared in reasonable yield starting with the analogous (4-chlorophenyl)phenyldimethox-methane. By using a three-fold excess of the Grignard reagent over $^{14}CO_2$ and a sodium hydroxide extraction of the hydrolyzed reaction mixture, a nearly quantitative conversion of $^{14}CO_2$ to product occurred. Additionally, since no other organic acid can be produced in the reaction, the product has the same level of radioactivity as the starting $Ba^{14}CO_3$. Finally, because the radioactivity was introduced in essentially the final step, a minimum of manipulations involving radioactive material was required.

EXPERIMENTAL

Materials and Methods. 4-Bromobenzophenone was purchased from Aldrich Chemical and $Ba^{14}CO_3$ from ICN (lot no. 422676, 56.3 mCi/mmol). PMR spectra were obtained on a Varian EM360, IR spectra on a Beckman Acculab, and UV-VIS spectra on a Cary 14.

(4-Bromophenyl)phenyldichloromethane (1). The basic procedure of Kekule and Franchimont (14) was used. Phosphorus pentachloride (40.0 g, 0.192 mol) and 4-bromobenzophenone (50.0 g, 0.191 mol) were mixed together as solids and heated in an oil bath at 140°C in a distillation type apparatus. After the reaction mixture melted and was thoroughly mixed, a vigorous reaction was initiated. Phosphorus oxychloride distilled over and was collected. The reaction was controlled by periodic cooling in a water bath. After the initial vigorous reaction subsided ($\sim$ 10 min), the reaction was maintained at 140-145°C with the oil bath and stirring for about 2 h until phosphorus oxychloride production ceased. During the latter stages of the

heating, a brown gas formed in small quantities, probably bromine gas from decomposition of the aromatic bromide. The product was obtained by vacuum distillation, 134-138°C/0.1 mm, 50.7 g (85%) yield. PMR and IR spectra were consistent with the expected spectra for the product. The UV spectrum of 1 in acetonitrile showed a 233 nm absorption max (ε_M = 13,400) and a small shoulder at 270 nm.

4-Bromobenzophenone dimethylketal (2). The procedure developed by MacKenzie (15) for the preparation of benzophenone dimethylketal was utilized. To a cooled solution of sodium methoxide [sodium metal (4.6 g, 0.20 mol) previously refluxed in 75 ml absolute methanol] was added (4-bromophenyl)-phenyldichloromethane (31.6 g, 0.10 mol) and stirred for about 2 h at about 10°C until a vigorous reaction was initiated. The reaction was controlled with cooling in an ice bath after which it was heated to reflux for 3 h. Diethyl ether (100 ml) was added to the reaction to precipitate sodium chloride, which was removed by filtration and thoroughly washed with fresh ether. The combined ether solution was dried over sodium sulfate and the supernatent solvent removed by rotary evaporation to yield a clear, off-white, viscous liquid. Vacuum distillation of this liquid at 117-120°C/0.1 mm yielded 25.5 g (83%) of clear, colorless product (2). PMR (CCl_4) spectra were consistent with the expected product and the methyl singlet (3.05 δ) to aromatic multiplet (7.09-7.52 δ) integration ratio was 1.9/3.0 versus the expected 2/3 ratio. The IR spectrum was also consistent with the expected structure of 2 and the UV spectrum in acetonitrile showed an absorption max at 228 nm (ε_M = 14,600) with a shoulder at 258 nm.

[1-^{14}C-Carboxy]-4-benzoylbenzoic Acid (4). Two 5 ml teflon stoppered vacuum reaction tubes (Pierce Chemical Co., Cat. No. 29560) were utilized in the radiochemical synthesis. In a separate vessel the Grignard reagent was prepared under Ar gas using 4-bromobenzophenone dimethyl ketal (3.0 g, 10 mmol), magnesium turnings (0.55 g, 20 mmol) and 25 ml of dry diethylether. Ten percent of this solution (1 mmol, 2.5 ml) was added under Ar to one of

the vacuum reaction tubes (A) which contained a small magnetic stirring bar. Tube A was linked to tube B via their side arms with a T connection containing a vacuum stoppered valve leading to a vacuum source. The Grignard solution in tube A was frozen with dry ice/acetone and tube A and the connected side arms of A and B evacuated to 0.1 mm. $Ba^{14}CO_3$ (64.9 mg, 0.325 mmol) was placed in the bottom of the other reaction tube (B) and 0.3 ml of concentrated H_2SO_4 was added carefully to the side of the top of the tube, frozen in place with liquid N_2, the tube evacuated to 0.1 mm and the stopper closed. The H_2SO_4 was allowed to melt and react with the $Ba^{14}CO_3$. The system was isolated from the vacuum and the teflon stopper in B was opened and the $^{14}CO_2$ present in B frozen out into A with liquid N_2. The stopper in A was closed and the reaction mixture was stirred magnetically until all the $^{14}CO_2$ had reacted. This reaction can be monitored by placing a plastic sleeve over the bottom end of tube A, inverting and adding liquid N_2 to the sleeve. Any remaining $^{14}CO_2$ will rapidly condense at the tip of the tube and the progress of reaction can be easily determined. In addition, this procedure forced the $^{14}CO_2$ to pass up and through the Grignard reagent solution when tube A was inverted. The overall reaction took about 30 minutes. Alternatively, a vacuum apparatus modeled on the one used by Dauben and co-workers for $^{14}CO_2$ Grignard reactions may be employed (16). The procedure described, however, uses readily available, simple glassware and gave excellent results. The reaction solution was then transferred to a 25 ml flask and the ether allowed to evaporate. The original reaction vessel was washed two times with 1 M HCl in dioxane (5 ml) and the wash transferred to the solid in the 25 ml flask. The flask was warmed to about 70°C with stirring for 2 h to hydrolyze the unreacted Grignard reagent (3), and the precursor of the product (4). The reaction mixture gradually turned a straw yellow color. The HCl-dioxane was removed by rotary evaporation, leaving a light yellow solid which was dissolved in ether, washed with 1 M HCl and then twice extracted with 1 M sodium hydroxide. The sodium hydroxide extractions were combined and acidified with 6 M HCl to yield an off-white solid. The solid was collected

by centrifugation and washed twice with 1 M HCl. The solid was then recrystallized using a minimum quantity of dioxane/water. The [1-^{14}C-carboxy]-4-benzoylbenzoic acid (4) was isolated as white needle-like crystals, 0.0723 g (98%). The theoretical specific radioactivity of the starting $Ba^{14}CO_3$ was 125,000 cpm/nmol and the observed specific radioactivity of the product was 126,800 cpm/nmol. A parallel synthesis using nonradioactive $BaCO_3$ gave a product that was identical to a commercial sample of 4-benzoylbenzoic acid (Aldrich Chemical) in melting point (including mixed melting point 196-197° unc.), UV, IR, and PMR spectra. The UV spectrum showed a 264 nm absorption maximum (ε_M = 20,000) in 0.01 M NaOH solution.

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