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A Simple Synthesis of $^{13}C_6$-Labelled Flavone and 5-Methoxyflavone

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SUMMARY

The $^{13}C_6$-labelled molecules, flavone and 5-methoxyflavone, with the carbon-13 label at all six carbons of the aromatic B ring, have been prepared for use as internal standards in isotope dilution-mass spectrometry. The key step involves addition of a labelled benzoyl group to the methyl group of a hydroxyacetophenone, forming a 1,3-diketone. Overall yields from $^{13}C_6$-benzoic acid were 38% for the labelled flavone and 45% for the labelled 5-methoxyflavone.

Keywords: carbon-13, 5-methoxyflavone, flavone, flavonoid, benzoic acid, isotope dilution-mass spectrometry.

INTRODUCTION

Isotope dilution-mass spectrometry is becoming an increasingly important method for the precise and accurate quantitative analysis of organic materials in biological matrices (1). In this technique, an isotopically-labelled version of the analyte, referred to as the internal standard, is added to the biological sample at an early stage of the analysis. This internal standard serves to account for loss of the substance of interest during the pretreatment phase, prior to mass spectrometry. In order to avoid complications caused by natural abundance isotopes, it is most

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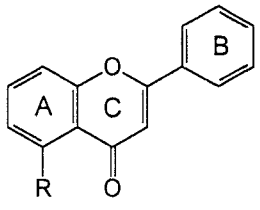
desirable for the internal standard to have a molecular weight that is at least three mass units higher than that of the compound under investigation (2).

Recently, we wished to prepare an internal standard for isotope dilution-mass spectrometry analysis of the flavonoid molecule, 5-methoxyflavone **1**. Our goal was to prepare a compound in which all six carbon atoms of the aromatic B ring were labelled with carbon-13. For reference and comparison, we also wished to synthesize a similar $^{13}C_6$-analog of the parent compound, flavone, **2**. In this report, we describe a simple and convenient synthesis of these two labelled flavones, starting from commercially available $^{13}C_6$-benzoic acid.



1 R = OCH_3
2 R = H

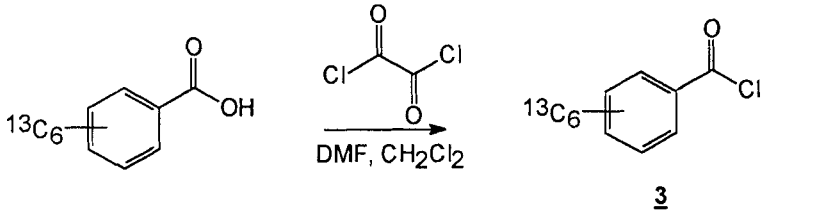
RESULTS AND DISCUSSION

There are currently a number of methods available to synthesize flavones (3), but perhaps the most common method is known as the Baker-Venkataraman sequence (4,5). In this process, a hydroxyacetophenone is treated with an aromatic acid chloride to form a benzoyl ester. This material is then treated with base to effect an acyl group migration to carbon, forming a 1,3-diketone, which is subsequently cyclized in acid to form the flavone. We have recently described a modified Baker-Venkataraman procedure whereby potassium t-butoxide is utilized to transform the hydroxyacetophenone into the 1,3-diketone in one pot (6). This

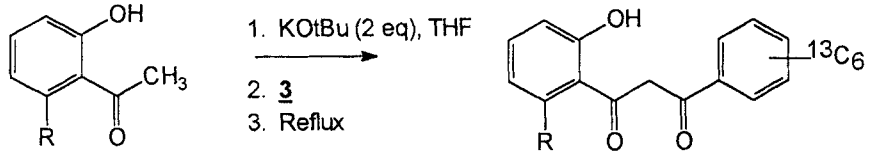
process was utilized successfully to prepare, in a convenient way, several hundred grams of 5-methoxyflavone (6). We hypothesized that a similar approach might also serve as a useful strategy for the synthesis of the labelled compounds. Therefore, we decided to assess the feasibility of adapting this approach to the synthesis of the desired labelled molecules.

Our results are indicated in Scheme 1. Treatment of commercially-available $^{13}C_6$-benzoic acid with oxalyl chloride in methylene chloride, in the presence of a catalytic amount of dimethylformamide, led to generation of crude $^{13}C_6$-benzoyl chloride **3**. The starting acetophenone, either

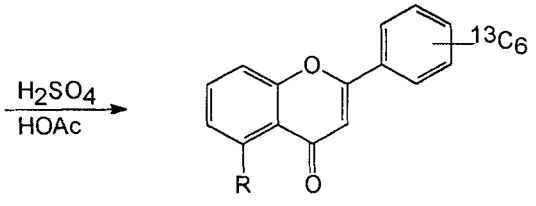
SCHEME 1



3



4 R = OCH_3
5 R = H



1 R = OCH_3 (45%)
2 R = H (38%)

hydroxymethoxyacetophenone **4** (7) or hydroxyacetophenone **5**, was treated with slightly more than two equivalents of potassium t-butoxide in tetrahydrofuran, and to this mixture was added labelled benzoyl chloride **3**. After heating, the crude diketones were isolated and cyclized directly with sulfuric acid in acetic acid. We were delighted to discover the process worked exceedingly well, producing labelled flavones **1** and **2** in 45% and 38% yield respectively, following flash chromatographic purification. It is interesting to note that the proton NMR spectra of these compounds revealed the expected 160 Hertz coupling between the aromatic protons and the carbon-13's to which the protons are attached. These somewhat broad splitting patterns are superimposed upon the typically sharp aromatic signals of the protons in the A ring.

In summary, $^{13}C_6$-labelled flavone and 5-methoxyflavone have been synthesized for use as internal standards in isotope dilution-mass spectrometry, in 38% and 45% yield, respectively. The key step employed a potassium t-butoxide-mediated synthesis of a labelled 1,3-diketone, which was subsequently cyclized in acid to the desired flavone molecules.

EXPERIMENTAL

Liquid chromatography was performed using flash chromatography conditions (8) with 50 x 60 micron silica gel purchased from Amicon of Beverly, Massachusetts, USA. Tetrahydrofuran (THF) was dried over sodium/benzophenone. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded using deuterated chloroform as a solvent. Melting points were uncorrected. $^{13}C_6$-benzoic acid (99%) was obtained from MSD Isotopes of Montreal, Canada.

Synthesis of $^{13}C_6$-Labelled 5-Methoxyflavone (1).

$^{13}C_6$-benzoic acid (284 mg, 2.22 mmol) was dissolved in methylene chloride (4 mL) and oxalyl chloride (352 mg, 0.242 mL, 2.77 mmol)

was added by syringe. To this stirred solution was added dimethyl formamide (16 mg, 0.017 mL, 0.22 mmol) via syringe, resulting in gas evolution and a vigorous reaction. The mixture was stirred at room temperature for 3 hours, and the yellowish solution was concentrated to a residue by rotary evaporation. Methylene chloride was added, and the solution was concentrated to dryness once more. This methylene chloride addition/evaporation process was repeated two more times, and the resulting amber oil of crude $^{13}C_6$-benzoyl chloride **3** was used immediately in the next step.

Into a two-necked 25 mL pear-shaped flask was placed potassium t-butoxide (547 mg, 4.88 mmol) in THF (5 mL). The suspension was cooled in a salt-ice bath and hydroxymethoxyacetophenone **4** (368 mg, 2.22 mmol) (7) was added, resulting in a yellow suspension. The suspension was stirred in the ice bath for 20 minutes and the above benzoyl chloride **3** in THF (4 mL) was slowly added. The mixture was stirred at 0 ^{o}C for 10 minutes and at room temperature for 45 minutes during which time it turned to a browner color. The reaction mixture was then refluxed for 8 hours and stirred overnight at room temperature. The mixture was subsequently acidified with 1N hydrochloric acid, forming two layers, which were then partitioned between water (20 mL) and methylene chloride (20 mL). The layers were separated, and the aqueous layer was further extracted with methylene chloride (2 x 20 mL). The combined organic layers were washed with water, dried (Na_2SO_4) and evaporated to a brown oil of crude diketone which was used immediately as such in the next step.

The above diketone was dissolved in glacial acetic acid (8 mL) and 3 drops of concentrated sulfuric acid were added. The reaction mixture was refluxed for 2 hours during which time it turned greenish-black. The mixture was then cooled to room temperature

and poured into ice water (35 mL), resulting in an aqueous mixture which was extracted with methylene chloride (3 x 25 mL). The combined organic layers were washed with water, half-saturated sodium bicarbonate solution, and brine. Drying (Na_2SO_4) and evaporation afforded a green oil which solidified. Purification by flash chromatography (8) with 40% hexane in ethyl acetate afforded the labelled 5-methoxyflavone 1 as a white solid (260 mg, 45% yield overall). mp 120-121 °C; ^{1}H NMR (300 MHz) δ 4.03 (s, 3 H, OCH_3), 6.77 (s, 1 H, H-C-3), 6.87 (d, 1 H, J = 10 Hz), 7.17 (d, 1 H, J = 10 Hz), 7.53 (br d, 3 H, H-^{13}C-3' and H-^{13}C-4', J = 160 Hz), 7.60 (d of d, 1 H, H-C-7), 7.93 (br d, 2 H, H-^{13}C-2', J = 160 Hz); MS (e.i.): m/z 258.

Synthesis of $^{13}C_6$-Labelled Flavone (2).

Flavone 2 was prepared from hydroxyacetophenone 5 in essentially the same manner as that described for 5-methoxyflavone 1, with two exceptions. First, in the diketone synthesis stage, the reaction was refluxed for 3 hours and worked up immediately after cooling to room temperature. Secondly, 10% ethyl acetate in hexane was utilized in the flash chromatographic purification of the final flavone molecule. The overall yield was 38%. mp 58-60 °C (softens at 52 °C); ^{1}H NMR (300 MHz) δ 6.87 (s, 1 H, H-C-3), 7.46 (d of d, 1 H, H-C-6), 7.58 (d, 1 H, H-C-8, J = 10 Hz), 7.60 (br d, 3 H, H-^{13}C-3' and H-^{13}C-4', J = 160 Hz), 7.70 (m, 1 H, H-C-7), 7.95 (br d, 2 H, H-^{13}C-2', J = 160 Hz), 8.29 (s, 1 H, H-C-5, J = 9 Hz); MS (e.i.): m/z 228.

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