Protocols and Methods

Journal of Labelled Compounds and Radiopharmaceuticals

Received 20 October 2013, Revised 10 November 2013, Accepted 12 November 2013 Published online 10 January 2014 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.3172

Synthesis of [7-^{14}C]bergapten

Crist N. Filer* and Thomas Rodgers

Bergapten (1) is a furocoumarin natural product and currently employed to treat skin disorders. Since past attempts to radiolabel 1 with ^{14}C were limited to only its 5-methoxy group, a synthesis of the required ring [7-^{14}C]1 is now described. The literature reported precursor 4-methoxy-6-hydroxybenzofuran-5-carboxaldehyde (3) was Wittig reacted with stabilized [carbonyl-^{14}C]methoxycarbonylmethylenetriphenylphosphorane (4) to obtain [7-^{14}C]1 in 47% radiochemical yield, with the desired product being characterized by thin-layer chromatography, HPLC, m.p. and proton NMR.

Keywords: Bergapten; carbon-14; furocoumarin; Wittig reaction

Introduction

The natural product bergapten (**1**) is a member of the furocoumarin (psoralen) family and easily obtained from numerous plant sources, many of which are still utilized in traditional Chinese medicine. It and related toxic botanical substances are also classified as phytoalexins, exploited by some plants as defensive agents against pests such as fungi and insects.[1] In recent years, bergapten and other psoralens have also been successfully employed in photochemical therapy to effectively treat skin conditions such as psoriasis and vitiligo. Single crystal X-ray diffraction studies have elucidated the product of this photolysis as a cycloaddition adduct between the psoralen pyrone ring and the DNA base thymine, thereby disrupting pathogenic cellular function.[2] Also, quantum mechanical calculations have indicated that bergapten is one of the more efficient photosensitizers in its structural class.[3] Despite the biological interest in this substance, there appears to be just a single description of radiolabeling bergapten with ^{14}C and only at its peripheral 5-methoxy position.[4] For a particular study, we were required to embed ^{14}C in the bergapten pyrone ring and specifically at the 7-position carbonyl group. We now describe a rather efficient method to accomplish that.

Results and discussion

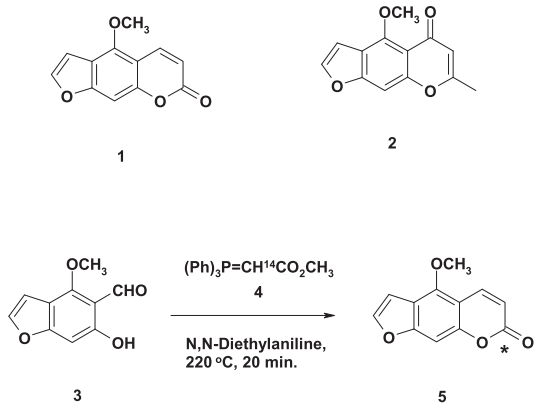
Because of its convenient isolation from diverse plant sources, there has been relatively little effort directed toward bergapten total synthesis over the past 60 years. Furthermore, few of these scattered reports provided a facile route, which could be applied to ^{14}C labeling. However, in reviewing the literature, one paper emerged with a useful strategy to both readily assemble the pyrone ring as well as access an appropriately functionalized furan containing unlabeled precursor. In 1970, Nour El-Din and Safwat reported the conversion of visnagin (**2**) to bergapten.[5] Visnagin, also a furocoumarin isolated from the flowering plant *Ammi visnaga*, has been utilized in Middle Eastern herbal medicine to treat kidney stones[6] and is commercially available. The authors described the four-step transformation of visnagin to key aldehyde intermediate **3**, starting with an oxidative pyrone ring scission and culminating in a McFadyen–Stevens (acylbenzenesulfonylhydrazide pyrolysis) aldehyde construction.[7] This route also appears to be the only published synthesis of aldehyde **3**. Conversion of intermediate **3** to bergapten was then accomplished by malonic acid condensation (with simultaneous pyrone ring closure) followed by decarboxylation.

The preparation of **3** worked just as described, quickly resulting in significant quantities of the aldehyde. However, for our ^{14}C synthesis, we decided to modify the published path to bergapten for several reasons. Not only was malonic acid (the potential ^{14}C reagent) used in large excess, but unless both of its carbonyls were radiolabeled, the resulting product specific activity would be significantly diluted as well. Utilizing such a [dual carboxyl-^{14}C]malonic acid would be both tedious to prepare and ultimately result in loss of volatile [^{14}C]carbon dioxide at the final (decarboxylation) step. To circumvent these complications, our plan was instead to treat aldehyde **3** with a ^{14}C-labeled stabilized Wittig ylide followed by concomitant lactone ring closure in a single step. Recognizing that a mixture of intermediate olefin geometrical isomers would likely form during the Wittig reaction, we employed refluxing diethylaniline to thermally promote ring closure.[8] As encouraging precedent for this Wittig reaction strategy was our prior successful synthesis of structurally related [3-^{14}C]coumarin from 2-hydroxybenzaldehyde using these same experimental conditions.[9] The synthesis of [7-^{14}C] bergapten (**5**) is displayed in Scheme 1. Wittig phosphorane **4** was prepared as described in the literature[10] and reacted with aldehyde **3** while monitored by thin-layer chromatography (TLC). After conventional workup, purification of **5** was accomplished by flash chromatography followed by recrystallization, giving extremely pure product with its HPLC, proton NMR and m.p. in exact agreement with that of authentic **1**. Although the radiochemical yield of 47% for the final step conversion of **3** to **5** can be termed modest, this small-scale transformation compared well with the

PerkinElmer Life Sciences & Technology Inc., 940 Winter Street, Waltham, MA 02451, USA

**Correspondence to: Crist N. Filer, PerkinElmer Life Sciences & Technology Inc., 549 Albany Street, Boston, MA 02118, USA.*
E-mail: crist.filer@perkinelmer.com

Journal of
Labelled Compounds and
Radiopharmaceuticals



Scheme 1. Synthesis of [7-14C]bergapten (**5**).

comparable two-step literature yield of 17% for **1** (based on malonic acid) and afforded pure mCi amounts of product **5**.

Experimental

General

All chemicals used were reagent grade. Evaporations were carried out on a Buchi rotary evaporator at bath temperatures less than 40 °C. Analytical and preparative TLC were performed on Analtech silica gel glass plates. Analytical HPLC was accomplished on a PerkinElmer instrument, and peak detection was performed simultaneously by UV and an IN/US Systems Beta RAM Model 3 radioactivity detector. Solution assays were performed with a PerkinElmer Tri-Carb 3100TR instrument. NMR spectra were obtained on a Bruker 300 MHz instrument and chemical shift values are expressed in parts per million (ppm) downfield from internal tetramethylsilane. M.p.s were done on a Thomas Hoover apparatus and are uncorrected.

4-Methoxy-6-hydroxybenzofuran-5-carboxaldehyde (3)

This intermediate aldehyde was prepared in comparable yield as previously reported[5] and recrystallized from methanol to give pale yellow crystals (m.p. 121–124 °C, lit[5] 125 °C); proton NMR ($CDCl_3$) δ 10.30 (s), 7.45 (s), 6.90 (s), 6.60 (s) and 4.25 ppm (s).

[Carbonyl-^{14}C]methoxycarbonylmethylenetriphenylphosphorane (4)

Precursor [1-^{14}C]bromoacetic acid (62 mg, 0.45 mmol, and 22.7 mCi) was dissolved in 10 mL of diethyl ether and treated with a diethyl ether solution of diazomethane until a yellow color persisted. The solution was stirred for 1 h at ambient temperature, and the diethyl ether was removed by rotary evaporation. The residue was dissolved in 5 mL of benzene to which was added triphenylphosphine (160 mg and 0.61 mmol). It was refluxed under nitrogen for 1 h, cooled to ambient temperature, and allowed to stir overnight. The solid that formed was filtered and washed with two 5 mL portions of diethyl ether. It was returned to the reaction flask to which 7 mL of benzene and 1 mL of water were added. The mixture was vigorously stirred with the addition of 0.35 mL of 2 M aqueous sodium hydroxide solution, and stirring was continued under nitrogen at ambient temperature for 1 h. The reaction was diluted with 10 mL of diethyl ether. The organic layer was separated, dried over sodium sulfate, filtered and evaporated to afford **4** (125 mg, 0.37 mmol,18.7 mCi, and 82% radiochemical yield) as an off-white solid.

[7-^{14}C]Bergapten (5)

A solution of aldehyde **3** (80 mg and 0.42 mmol) and Wittig reagent **4** (125 mg, 0.37 mmol, and 18.7 mCi) in 4 mL of *N,N*-diethylaniline was heated at 220 °C under nitrogen, and the reaction was monitored by TLC (hexane : ethyl acetate [4:1]). After 20 min, the reaction was complete. It was cooled and diluted with 20 mL of diethyl ether and washed sequentially with 10 mL portions of water and 1 N aqueous hydrochloric acid. The ether layer was rotary evaporated to a small volume and purified by silica gel flash chromatography eluted with a solution of hexane : ethyl acetate (4:1). The progress of the flash purification was monitored by TLC (same system as previously mentioned) with liquid scintillation counting of elution fractions. Appropriate fractions were pooled and evaporated. The resulting residue was crystallized from 7 mL of methanol to afford 38 mg (m.p. 188–190 °C; lit[5] 188–191 °C) of **5** (8.8 mCi, 47% radiochemical yield based on **4**), which was 99% radiochemically pure on TLC (hexane : ethyl acetate [4:1]) as well as reverse phase HPLC (eluted with acetonitrile : water (1:1)). Product **5** also coeluted with authentic **1** in both of these chromatographic systems. The specific activity of **5** was measured to be 50.5 mCi/mmol by gravimetric assay. Proton NMR ($CDCl_3$) δ 8.17 (d, 1, J = 9.5 Hz), 7.60 (d, 1, J = 2 Hz), 7.14 (s, 1), 7.03 (d, 1, J = 2 Hz), 6.28 (d, 1, J = 9.5 Hz), and 4.26 ppm (s, 3).

Acknowledgement

We would like to acknowledge the contribution of Dr. Puliyer Srinivasan of PerkinElmer Life Sciences & Technology in obtaining the NMR spectra.

Conflict of Interest

The authors did not report any conflict of interest.

References

[1] M. Berenbaum, P. Feeny, *Science* **1981**, *212*, 927–929.
[2] C. E. Pfluger, R. L. Ostrander, *Photochem. Photobiol.* **1989**, *49*, 375–379.
[3] J. J. Serrano-Perez, R. Gonzalez-Luque, M. Merchan, L. Serrano-Andres, *J. Photochem. Photobiol. A: Chem.* **2008**, *199*, 34–41.
[4] L. Musajo, G. Rodighiero, A. Breccia, F. Dall'Acqua, G. Malesani, *Photochem. Photobiol.* **1966**, *5*, 739–745.
[5] H. M. Nour El-Din, H. M. Safwat, *U. A. R. J. Pharm. Sci.* **1970**, *11*, 45–51.
[6] K. G. Haug, B. Weber, G. Hochhaus, V. Butterweck, *Eur. J. Pharm. Sci.* **2012**, *45*, 79–89.
[7] J. S. McFadyen, T. S. Stevens, *J. Chem. Soc.* **1936**, 584–587.
[8] K. C. Majumdar, S. Alam, *J. Chem. Res.* **2006**, *5*, 281–285.
[9] C. N. Filer, T. Rodgers, unpublished results.
[10] E. S. Monteagudo, G. Burton, E. G. Gros, *Helv. Chim. Acta* **1990**, *73*, 2097–2100.

