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Optimization and Synthesis of (*E*)-4-[2-(3,4-Dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-y1)-1-propenyl]benzoic Acid-11-[^{14}C]

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SUMMARY

Heteroarotinoids may be useful in the treatment of skin disorders and a wide variety of cancers. A synthesis of the C-14 labelled heteroarotinoid, (*E*)-4-[2-(3,4-dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-y1)-1-propenyl]benzoic acid-11-[^{14}C] (***1**) is described via a multistep procedure similar to that used to obtain the unlabelled compound **2**. The latter has shown good activity in several assays compared to the standard *trans*-retinoic acid (**3**). Reduction of the carbonyl group in 4,4-dimethylchroman-6-y1 methyl ketone-(carbonyl-^{14}C) (***5**) with $LiAlH_4$ gave alcohol ***6**. Phosphorylation with triphenylphospine hydrobromide in methanol led to the corresponding phosphonium salt ***7**. Addition of *n*-butyllithium to ***7** in ether at -78 °C generated the Wittig reagent *in situ* and to this was added ethyl 4-formylbenzoate. Workup and chromatography afforded *E*-ester ***8** and *Z*-ester ***9** which were both hydrolyzed to labelled ***1**. Labelled ***1** was identical to unlabelled **2** in terms of spectral data and melting point. The specific activity of ***1** was determined to be 57.2 μCi/mg.

KEY Words: (*E*)-4-[2-(3,4-Dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-y1)-2-propenyl]-benzoic acid-11-[^{14}C], heteroarotinoid, reduction, Wittig condensation, anticancer agent.

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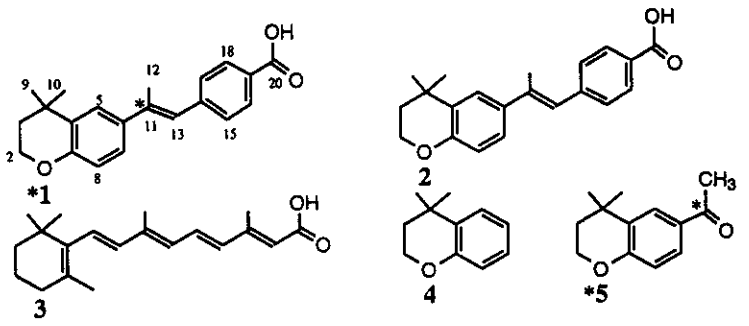
INTRODUCTION

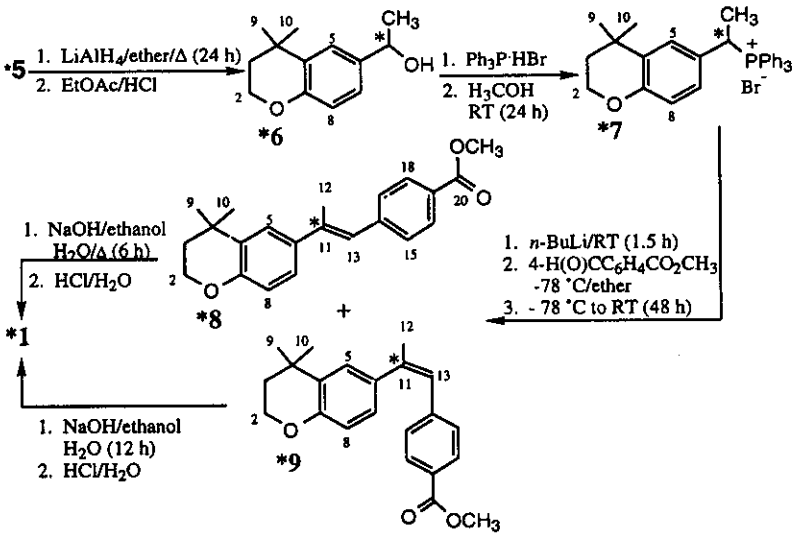
Retinoids are natural and synthetic analogs of vitamin A and are involved in the regulation of several biological functions including cell differentiation and proliferation. The functions involve binding to nuclear receptors which regulate gene transcription. The six known retinoid receptors are divided into two families, namely, the retinoic acid receptor family (RARα, -β, -γ) (1) and the retinoid X receptor family (RXRα, -β, -γ) (2). Clinically, retinoids are used to treat skin disorders and certain cancers and are currently being investigated in several other therapeutic areas, including arthritis, dyslipidemias, and the prevention of HIV-induced lymphopenia (3-5). Many natural and synthetic retinoids are of limited therapeutic value because of undesirable side effects such as mucocutaneous toxicity and teratogenicity (6). Heteroarotinoids possess an aryl ring and at least one heteroatom in a ring system (7). Heteroarotinoids have exhibited marked activity in the tracheal organ culture (TOC) assay (8-9), the ornithine decarboxylase (ODC) assay (8-10), and, to a limited degree, in the differentiation of HL-60 cells (10). Labelled ***1** is important since in the ODC assay (8-9), (*E*)-4-[2-(3,4-dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-y1)-1-propenyl]benzoic acid (**2**) exhibited excellent activity compared to the standard *trans*-retinoic acid (**3**). Since disposition studies of these compounds in animals are of interest, C-14 labelled ***1** was prepared. An assessment of the metabolism of *trans*-retinoic acid (**3**), as well as that of natural and synthetic retinoids (11-12), suggested the C-14 label be at the 11-position in ***1**.

RESULTS AND DISCUSSION

The key C-14 labelled ketone ***5** (42.7 mCi/mmol) was obtained from Sigma Radiochemicals (P.O. Box 14508, St. Louis, MO 63178) from cold **4** (8). Reduction of ketone ***5** proceeded well with $LiAlH_4$/ether and gave a good yield (85%) of alcohol ***6**. The workup required careful destruction of excess $LiAlH_4$ with ethyl acetate below 5 °C in order to prevent dehydration of the benzylic alcohol to the corresponding alkene. Previous experience with secondary and tertiary alcohols in related chroman systems had demonstrated a tendency for the latter to undergo elimination under less than neutral



conditions as exist in the decomposition of the $LiAlH_4$. Stirring a suspension of triphenylphosphine hydrobromide and labelled alcohol *6 in methanol at RT for 24 h gave a clear oil which was triturated with dry ether to produce the white, labelled salt *7



with a melting point [152-158 °C (8)] higher than previously reported for unlabelled 7 [149-155 °C (8)]. Recrystallization of labelled *7 gave a sample with a mp of 214-216 °C. All spectral data for labelled *7 were consistent with previous literature values for cold 7 (8). This confirmed the structure of 7 and is the purest sample reported to date.

A Wittig reagent of the anion from salt *7 was generated in dry ether at RT with 1.5 equivalents of *n* -BuLi in hexane. The resulting solution was cooled to -78 °C and was treated with a solution containing 1.4 equivalents of methyl 4-formylbenzoate in dry

ether. The yellow oil produced was subjected to flash chromatography over silica gel [hexane:ethyl acetate (90:10)]. The *E*-ester ***8** was obtained as a solid (44%) by recrystallization (95% ethanol). The crude *Z*-ester ***9** was obtained as a thick oil (37%).

It is well known that some *Z*-isomers can be converted to *E*-isomers at high temperature (13). To increase the yield of *E*-acid ***1**, it would be useful if the *Z*-ester ***9** could be converted to the *E*-acid ***1**. Since the last reaction was to be done at reflux in ethanolic NaOH, it was reasoned that the *Z*-ester ***9** might be directly converted to *E*-acid ***1**. This saponification of labelled *Z*-ester ***9** led to the labelled *E*-acid ***1**. The spectral data for ***1** matched with literature values for unlabelled *E*-acid **2**. Structural confirmation of labelled *E*-acid ***1** was based on ^{1}H NMR, ^{13}C NMR, and ultraviolet (UV) analyses in comparison to data in the literature for **2** (7, 8).

EXPERIMENTAL

General Information. All reactions were carried out at or near room temperature (RT), with a a magnetic stirrer, an under N_2 unless otherwise noted. During workup, solvents were removed with a rotary evaporator unless otherwise stated. IR spectra were recorded on a Perkin-Elmer 200 FT-IR as films or as KBr pellets. All ^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova-400 BB spectrometer operating at 399.99 MHz and 100 MHz, respectively, and the signals were referenced to TMS. Melting points were determined with a Thomas-Hoover melting point apparatus and were uncorrected. All commercial reagents and reagent grade solvents were used without further purification unless otherwise stated. Alcohol ***6** was prepared by the known procedure from 4,4-dimethylchroman (**4**) using C-14 labelled acetyl chloride (^{14}C=O) as the co-reagent (8).

3,4-Dihydro-4,4-trimethyl-2*H*-1-benzopyran-6-methanol-11-[^{14}C] (***6**)

A solution of the 4,4-dimethylchroman-6-yl methyl ketone-(carbonyl-^{14}C) (***5**, 535.5 mg, 2.62 mmol, 42.7 mCi/mmol) in dry ether (5 mL) was added dropwise (15 min) to a stirred suspention of $LiAlH_4$ (168 mg, 4.43 mmol) in dry ether (5 mL). The mixture, a grey suspension, was heated at reflux for 24 h. After the solution was cooled to RT (1 h), ethyl acetate (2 mL) was slowly added to destroy excess $LiAlH_4$ (an ice bath with

temperature below 5 °C). A solution of HCl (5%, 4 mL) was then added slowly, and the resulting grey suspention was stirred (15 min). Ether (20 mL) was added, and the resulting aqueous layer was separated. The aqueous layer was extracted with ether (4 x 20 mL), and the combined organic phases were washed with saturated $NaHCO_3$ (3 x 20 mL), water (2 x 20 mL), and saturated brine (2 x 20 mL). After the solution was dried (Na_2SO_4, 8 h), the solvent was evaporated at RT followed by high vacuum (0.8 mm Hg) at 50-55 °C to give 460 mg (85%) of ***6** as a light yellow oil. All spectral data via IR, ^1H NMR, and ^{13}C NMR analyses matched the literature values for ***6** (8).

[1-(3,4-Dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-yl)ethyl]triphenylphosphonium Bromide-11-[^{14}C] (***7**)

A solution of alcohol ***6** (450 mg, 2.18 mmol) in anhydrous H_3COH (18 mL) and triphenylphosphonium hydrobromide (900 mg, 2.62 mmol) was stirred at RT (24 h). The solvent was evaporated, and the resulting clear oil was triturated repeatedly with dry ether until solidification occurred. The resulting white solid was suspended with stirring in dry ether overnight. After filtration, the white solid ***7** was recrystallized (CH_2Cl_2:EtOAc; 1:4), filtered, and dried at high vacuum (0.8 mm Hg) at 100 °C for 2 h to give 880 mg (76%) of ***7**; mp 214-216 °C, [lit (8) 149-155 °C]. All ^1H NMR and ^{13}C NMR data for ***7** matched those of cold **7** in the literature (8).

Methyl (*E*)-4-[2-(3,4-Dihydro-4,4-dimethyl-2*H*-1-benzopyran-6-yl)-1-propenyl]benzoate-11-[^{14}C] (***8**)

A solution of *n*-butyllithium in hexane (10 *M*, 0.31 mL, 3.1 mmol) was added dropwise to a stirred suspension of labelled salt ***7** (720 mg, 1.355 mmol) in dry ether (5 mL). The mixture changed from a white suspension to yellow to a dark red suspension. The solution was stirred (1.5 h). The resulting dark, clear, homogenous solution was cooled to -78 °C (Dry Ice/acetone), and a solution of methyl 4-formylbenzoate (400 mg, 2.44 mmol) in dry ether (10 mL) was added dropwise (15 min). The reaction mixture changed from a dark-reddish color to a brown color. The mixture was stirred (10 min) at -78 °C, and then the Dry Ice/acetone bath was removed. After 12 h, the reaction mixture was a light brown color. After 72 h, the solvent was suctioned filtered. The

resulting solid was washed with dry ether (50 mL), and the filtrate was concentrated to give 790 mg of a thick yellow oil. This yellow oil was subjected to flash chromatography over silica gel [hexanes:EtOAc (100:1)]. The combination of fractions 24-30 (2 mL/min drop rate; 5 mL fraction size) gave labelled *E*-ester ***8** as a light yellow, viscous oil. The oil was dissolved in a minimum amount of boiling 95% ethanol and filtered hot. Concentration of the filtrate and cooling the remaining solution to RT gave 200 mg (44%) of labelled (*E*)-isomer ***8** as small white, needles; mp 89-90.5 °C [lit (8) mp 90-90.5 °C]. All IR, ^{1}H NMR, and ^{13}C NMR data matched previous literature values for cold **8** (8). The combination of fractions 16-23 gave 170 mg (37%) of (*Z*)-ester ***9** which, without further purification, was saponified to the *E*-acid ***1**. The properties of this sample of ***1** were identical to those of the ***1** obtained from ***8** (see below).

(*E*)-4-[2-(3,4-Dihydro-4,4-dimethyl-2*H*-benzopyran-6-yl-propenyl)]benzoic Acid-11-[^{14}C] (***1**)

To labelled ***8** (200 mg, 0.59 mmol) was added NaOH (104.5 mg, 2.62 mmol) in hot absolute EtOH (3 mL) and water (10 mL), and the resulting mixture was boiled (6 h, N_2). After 1 h at reflux, the mixture was a clear, colorless solution with some small suspended particles, but a clear solution formed after 6 h at reflux. After cooling slowly to RT, the solution was acidified [conc HCl, pH = 3]. The resulting white solid was filtered, washed with water, and air dried. Recrystallization (95% ethanol) gave 120 mg (63%) of acid ***1** as a tiny crystals; mp 179-180 °C [lit (8) 180-180.5 °C]. All spectral data (IR, ^{1}H NMR, ^{13}C NMR analyses) for ***1** matched literature values for cold **2** (8).

Thermal Conversion and Saponification of Labelled *Z*-ester ***9** to Labelled *E*-Acid ***1**

The *Z*-ester ***9** (160 mg, 0.48 mmol), NaOH (84 mg, 2.09 mmol) in hot absolute EtOH (3 mL) and water (10 mL) were boiled (12 h). After cooling slowly to RT, the solution was acidified [conc HCl, pH = 3]. The resulting white solid was filtered, washed with water, and air dried. Recrystallization (95% ethanol) gave 70 mg (46%) of *E*-acid ***1** as a crystalline solid, mp 179-180 °C. All spectral data for ***1** matched that of the known cold **2** (8). The specific activity of ***1** was determined on a 10 μL aliquot (prepared by dissolving a 7.3 mg sample in 4 mL of HPLC grade methanol) via the use of a TRI-CARB liquid scintillation analyzer (model 1900-CA, Packard Instrument Company,

Downers Grove, IL). The specific activity of acid ***1*** was 57.2 μCi/mg of sample. A mixture melting point determination of this material with an authentic sample of cold **2** did not show a depression (mp 179-180 °C). A purity check of the final product ***1***, via TLC analysis with three separate solvent systems (hexanes:EtOAc:HOAc, 3:3:0.2; ether:HOAc, 6:0.2, and benzene:HOAc, 5:0.2), revealed only one spot. An HPLC analysis (Waters 6000-A unit) of ***1*** with a Whatman C18 column and 1:1 gradients of H_3COH:H_2O and H_3COH:HOAc showed only one peak

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