SYNTHESIS OF CARBON-14 LABELLED XENALIPIN – A POTENTIAL HYPOLIPIDEMIC AGENT

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SUMMARY

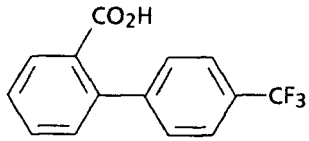
Xenalipin 1 (4'-trifluoromethyl-2-biphenylcarboxylic acid) was synthesized in the [^{14}C]-labelled form with specific activity 21.0 mCi/mmol suitable for metabolism and distribution studies in animals. The synthetic sequence involved conversion of [^{14}C]-benzoic acid to the 4,4-dimethyl-2-oxazoline derivative, formation of an organozinc compound, palladium-catalyzed coupling of the arylzinc-oxazoline with 4-trifluoromethyliodobenzene, and acid hydrolysis. The radiochemical purity was 99.4%.

Key Words: xenalipin, 4'-trifluoromethyl-2-biphenylcarboxylic acid, hypolipidemic agent.

INTRODUCTION

Epidemiological studies have strongly implicated elevated total plasma cholesterol as a major risk factor for coronary heart disease (1), and have shown that elevated plasma triglycerides can be positively correlated with the risk of the disease (2).

Xenalipin (1, 4'-trifluoromethyl-2-biphenylcarboxylic acid) has been shown to cause significant reductions in serum cholesterol and triglycerides in two animal species, and the results obtained suggest that xenalipin has a profile of activity which would be beneficial in therapy for hyperlipidemia (3).



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To facilitate extensive metabolism and distribution studies of xenalipin in animals, a carbon-14 labelled version of 1 was required. These studies required that the [^{14}C]-1 should have a high chemical

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and radiochemical purity, and a minimum specific activity of 20 mCi/mmol.

This paper describes the preparation of [^{14}C]-labelled 1 with specific activity 21.0 mCi/mmol.

RESULTS AND DISCUSSION

Methods for the synthesis of unlabelled 1 have been described by Bell, Burke, Hodgson and Shumaker (4), and by Eaddy (5). However, neither of these routes was suitable for the simple and efficient preparation of [^{14}C]-labelled 1. The overall yields were low, and formation of the necessary Grignard reagents was frequently problematical. Further, there are examples in the literature of violent explosions occurring during the formation of trifluoromethylphenylmagnesium bromides (6,7).

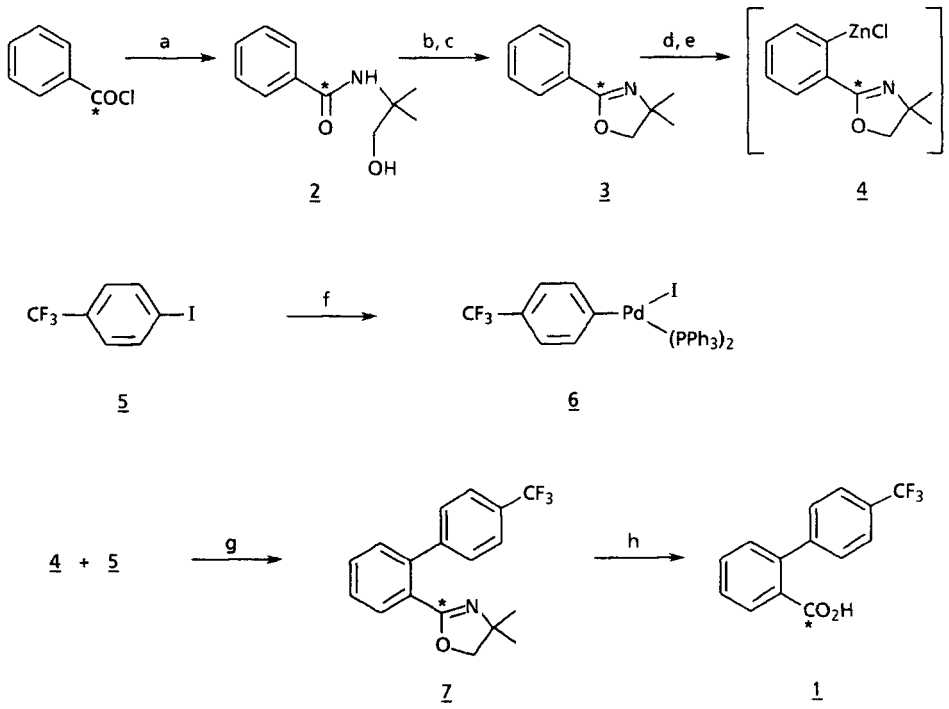
Further development work has produced a new reaction sequence which obviates the formation of the potentially hazardous trifluoromethylphenyl Grignard reagent, simplifies the work-up procedure, and gives a higher overall yield than either of the previous syntheses. This sequence utilizes a more readily available starting material (benzoic acid or benzoyl chloride) and has been used for the synthesis of [^{14}C]-1 from [^{14}C]-benzoic acid as shown in Scheme I.

The carboxylic acid group of benzoic acid was first masked as its 4,4-dimethyl-2-oxazoline derivative using a procedure developed by Meyers *et al* (8) for similar phenyloxazolines.

Benzoyl chloride (1 equiv.) in CH_2Cl_2 was added to a solution of 2-amino-2-methyl-1-propanol (2 equivs.) in CH_2Cl_2 at 0°C to give an assumed quantitative yield of *N*-(2-hydroxy-1,1-dimethylethyl)-benzamide 2. Cyclization in excess $SOCl_2$ at 0°C was followed by isolation of the hydrochloride salt, neutralization in aqueous NaOH solution, ether extraction and vacuum distillation to give 2-phenyl-4,4-dimethyl-2-oxazoline 3 as a colorless oil in 62.8% yield from benzoyl chloride.

Due to the strong *ortho*-directing effect of the oxazoline, metalation of 3 in tetrahydrofuran with one equivalent of 1.6*M* butyllithium in hexane at -25°C regioselectively gave the 2-lithioderivative (9), and this was converted to the 2-arylzinc chloride 4 by the addition of 1 equivalent of ~1*M* anhydrous zinc chloride in ether at -25°C. Without isolation, this intermediate monoarylzinc species 4 was used in a palladium(II)-catalyzed cross-coupling with 4-iodobenzotrifluoride 5 using the general mild procedure of Negishi (10), but using the catalyst iodo(4-trifluoromethylphenyl)-bis(triphenylphosphine)palladium(II) 6 (3 mole % based on 5) as used in unsymmetrical biphenyl formation by Sekiya and Ishikawa (11). Catalyst 6 was prepared in a separate step from tetrakis(triphenylphosphine)palladium(0) and 5 by the previously published procedure (5), which was based on the general method for the preparation of palladium(II) complexes developed by Fitton *et al* (12).

Scheme I



a) 2-amino-2-methyl-1-propanol, CH_2Cl_2
b) $SOCl_2$
c) NaOH
d) BuLi in hexane, THF, -25°C
e) anhydrous $ZnCl_2$ in ether, -25°C
f) $(Ph_3P)_4Pd(0)$, benzene
g) THF, 6; H_2O
h) 3*N* HCl; MeOH, 50% NaOH; CH_2Cl_2

Thus, the solution of 4 was treated with 1 equivalent of 5 in THF, and then with 0.03 equivalent of 6 and allowed to warm up to ambient temperature overnight. Quench and work-up gave the unsymmetrically substituted biphenyl oxazoline 7 as a yellow oil. Hydrolysis of 7 was achieved by a two-stage sequence in aqueous hydrochloric acid followed by saponification of the resulting amino-ester hydrochloride with aqueous sodium hydroxide in methanol, and the resulting mixture worked up to give 55.6% of crude biphenic acid 1 as a cream crystalline residue. Although the product was of excellent purity by TLC and HPLC, it was recrystallized from CH_2Cl_2 to give white crystalline 1 in an overall yield of 44.9%. The product was shown to be of excellent purity by reverse phase TLC, HPLC, ^1H NMR and melting point.

The radiolabelled synthesis of [^{14}C]-1 was carried out using essentially the same reaction conditions, and the details are included in the experimental section.

The preparation of [oxazoline-2-^{14}C]-3 from [carboxyl-^{14}C]-benzoic acid was performed by Wizard Laboratories, Davis, California. Elaboration of this material as described above provided a 61.0% yield of crude [^{14}C]-1.

Recrystallization of the crude [^{14}C]-1 from CH_2Cl_2 gave a 37.3% yield of [carboxyl-^{14}C]-1 with a specific activity of 21.0 mCi/mmol. The radiolabelled material was identical to an authentic sample of 1 by TLC, in a system which clearly separated 1 from benzoic acid and from valerophenone. The radiochemical purity was >99% by plate-scanning.

EXPERIMENTAL

[Oxazoline-2-^{14}C]-4,4-dimethyl-2-phenyl-2-oxazoline was obtained from Wizard Laboratories, Davis, California. 1.6*M* Butyllithium in hexane, benzoyl chloride and 2-amino-2-methyl-1-propanol were purchased from Aldrich Chemical Company. 0.872*M* Zinc chloride in diethyl ether was purchased from Alfa Products. 4-Iodobenzotrifluoride was purchased from Fairfield Chemical Company. Tetrakis(triphenylphosphine)palladium(0) was purchased from Strem Chemicals. All other solvents and reagents were of reagent purity and were obtained from readily available commercial sources. Tetrahydrofuran and benzene were dried over Type 4A Molecular Sieves. Thin layer chromatography (TLC) was performed on 5 x 20 cm 0.2 mm $KC_{18}F$ pre-coated reversed phase glass plates (Whatman), or on 5 x 20 cm glass plates pre-coated with 0.25 mm Silica gel 60 (E. Merck). Proton NMR spectra were obtained using a Perkin-Elmer R-24B spectrometer (60 MHz) with tetramethylsilane as internal standard. Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Radiochemical purity was determined on the TLC plate with a Bioscan System 200 Imaging Scanner. The specific activity was determined by counting an aliquot of a solution whose concentration had been determined by UV spectroscopy using a Packard Tri-Carb Minaxi 4000 liquid scintillation counter and Aquasol-2 (DuPont NEN) liquid scintillation cocktail.

2-Phenyl-4,4-dimethyl-2-oxazoline 3

To a stirred solution of 2-amino-2-methyl-1-propanol (178.28 g; 2 mol) in CH_2Cl_2 (750 mL) at 0°C was added dropwise a solution of benzoyl chloride (140.57 g; 1 mol) in CH_2Cl_2 (100 mL). After stirring overnight at 25°C, the white precipitate was filtered and washed with CH_2Cl_2. Evaporation of the combined CH_2Cl_2 solutions gave a quantitative yield of crude *N*-(2-hydroxy-1,1-dimethylethyl)benzamide 2 as a white sticky solid.

Thionyl chloride (235 mL; 3.3 mol) was added dropwise with stirring at 0°C. After 3 h stirring at 25°C, TLC (SiO_2) using $CHCl_3$/MeOH (4/1, v/v) showed complete conversion from amide 2 ($R_f = 0.6$; not detected) to oxazoline 3 ($R_f = 0.7$), and the solution was poured into ether (3 L) with stirring. Most of the ether was decanted and fresh ether (4 L) was added. The granular hydrochloride salt was filtered, washed with ether (3 x 200 mL), and added to a stirred solution of NaOH (60 g) in H_2O (1 L). The mixture was extracted with ether (3 x 300 mL), and the ethereal solution was washed with water (400 mL) and saturated NaCl solution (400 mL) leaving a dark yellow solution.

The solution was dried, treated with activated charcoal (Darco G-60), and filtered through Celite 545. Evaporation of the light yellow solution gave 139.84 g (79.8% yield) of crude oxazoline 3 as a yellow oil. Distillation under vacuum gave 121.35 g (62.8%) of oxazoline 3 as a colorless oil, b.p. 63-65°C / 0.15-0.25 mm [lit. (13) b.p. 124°C / 20 mm]; ^{1}H NMR ($CDCl_3$): δ 1.3 (s, 6 H, $C(CH_3)_2$), 4.0 (s, 2 H, CH_2), 7.35 (m, 3 H, Ar-H-3, -4 and -5), 7.9 (m, 2 H, Ar-H-2 and -6).

Iodo(4-trifluoromethylphenyl)-bis(triphenylphosphine)palladium(II) 6

To a slurry of tetrakis(triphenylphosphine)palladium(0) (12.61 g, 10.9 mmol) in dry benzene (35 mL) under nitrogen was added a solution of 4-iodobenzotrifluoride (2.97 g, 10.9 mmol) in benzene (35 mL). One hour after dissolution the solvent was evaporated and the residue triturated with cold diethyl ether. The iodo(4-trifluoromethylphenyl)-bis(triphenylphosphine)palladium(II) compound (9.58 g, 97%) was filtered, dried *in vacuo*, and stored in a brown bottle under nitrogen.

4'-Trifluoromethyl-2-biphenylcarboxylic acid 1

The procedure for preparation of 1 from 3 was essentially identical to that used below in the preparation of [^{14}C]-1. From 788.5 mg of 3 was obtained 538.3 mg (44.9%) of white solid 1; m.p. 168-169°C [lit. (5) m.p. 167-169°C]; ^{1}H NMR ($CDCl_3$): δ 7.2-7.7 (m, 7 H, Ar-H), 8.0 (m, H, H-3), 10.3 (bs, H, CO_2H); TLC (C_{18}) using MeCN/H_2O/HOAc (70/30/0.5, v/v), $R_f = 0.69$; HPLC using Alltech Spherisorb C_{18} 5 μ 4.6 mm x 25 cm column, MeOH/H_2O/HOAc (62/38/0.05, v/v), flow rate 1.0 mL/min, UV at 237 nm: $t_R = 16.4$ min.

[Carboxyl-^{14}C]-4'-Trifluoromethyl-2-biphenylcarboxylic acid 1

A stirred solution of [oxazoline-2-^{14}C]-4,4-dimethyl-2-phenyl-2-oxazoline 3 (811.8 mg with specific activity ~20 mCi/mmol) in dry THF (7.9 mL) at -25 to -30°C under argon was treated dropwise with 1.6*M* butyllithium in hexane (2.90 mL; 296.5 mg BuLi) and the orange mixture was stirred for 30 min. 0.872*M*

$ZnCl_2$ in ether (5.31 mL; 626.7 mg $ZnCl_2$) was added dropwise and the yellow mixture of 4 was stirred for 30 min. at -25 to -30°C. A solution of 4-iodobenzotrifluoride 5 (1.260 g) in dry THF (3.5 mL) was added dropwise followed by a solution of the Palladium(II) catalyst 6 (126.4 mg) in dry THF (3.6 mL). The mixture was stirred vigorously and the cooling bath was warmed up to room temperature during 2 h. After stirring overnight at 25°C, TLC (C_{18}) of an aliquot added to dilute NH_4OH and extracted with ether, using MeCN/H_2O (7/3, v/v), showed virtually complete conversion from oxazoline 3 ($R_f = 0.55$) to the intermediate biphenyloxazoline 7 ($R_f = 0.45$). The reaction mixture was quenched dropwise with H_2O (6.2 mL), and the mixture transferred to a separatory funnel together with H_2O (25 mL) and ether (25 mL). After separation, the aqueous layer was extracted with ether (3 x 20 mL). The combined ethereal solutions were washed with H_2O (20 mL) and evaporated to dryness under reduced pressure to give crude [^{14}C]-7 as a viscous yellow oil. H_2O (12.5 mL) and 12*N* HCl (4.2 mL) were added and the stirred mixture was refluxed under argon for 1.5 h. The cooled mixture was evaporated to dryness under reduced pressure. MeOH (11.4 mL) and 50% NaOH solution (4.65 mL) were added and the stirred mixture was refluxed under argon for 2 h. TLC (C_{18}) of an aliquot added to dilute HCl and extracted with ether, using MeCN/H_2O/HOAc (70/30/0.5, v/v), showed mainly 1 ($R_f = 0.69$) and two minor impurities. Most of the MeOH was evaporated under reduced pressure and the residual mixture transferred to a separatory funnel together with H_2O (110 mL) and ether (30 mL). After separation, the ether layer was extracted with H_2O (30 mL). The combined aqueous solutions were washed with ether (20 mL), acidified with 12*N* HCl to pH = 1 and concentrated under reduced pressure to ~30 mL volume. The mixture was transferred to a separatory funnel together with ether (50 mL) and 1*N* HCl solution (5 mL). After separation, the ether layer was washed with 1*N* HCl solution (20 mL) and H_2O (20 mL), dried and evaporated to dryness under reduced pressure to give crude [^{14}C]-1 as a pinkish-white solid (752.0 mg; 61.0% weight yield). CH_2Cl_2 (6.5 mL) was added, the mixture heated until complete solution was attained, then evaporated under reduced pressure to a volume of ~4 mL, and cooled at 0°C overnight.

The crystals were filtered, washed with ice-cold CH_2Cl_2 (1 mL), and dried *in vacuo* at 56°C overnight. The yield of white crystalline solid [^{14}C]-1 was 460.3 mg (36.3 mCi; 37.3% yield from 3) with specific activity 21.0 mCi/mmol.

TLC (C_{18}) using MeOH/0.1*M* HOAc (65/35, v/v) showed single-spot material with $R_f = 0.48$ corresponding to authentic 1. Radioactive chromatogram scanning showed the presence of 0.6% impurity at $R_f = 0.74$ corresponding to benzoic acid, but valerophenone ($R_f = 0.13$) was not detected; thus the radiochemical purity was >99%.

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