SYNTHESIS OF ^{14}C OR ^{3}H-LABELLED INDOMETACIN FARNESIL (E-0710)

Shinya Abe*, Isao Yamatsu, Chihiro Yamato, Seiichi Kobayashi and Mannen Mishima
Tsukuba Research Laboratories, Eisai Co., Ltd. 1-3 Tokodai 5-chome, Tsukuba-city, Ibaraki 300-26 Japan

SUMMARY

A ^{14}C or ^{3}H-labelled, 7:3 (2E:2Z) isomeric mixture of 6E-3,7,11-trimethyl-2,6,10-dodecatrienyl-1-(p-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetate (indometacin farnesil; IMF), a prodrug of indomethacin, was synthesized in order to study the pharmacokinetic profiles of this drug. Esterification of a 7:3 (2E:2Z) isomeric mixture of 6E-3,7,11-trimethyl-2,6,10-dodecatrienol [farnesol (3)] with [2-^{14}C] indomethacin [^{14}C-IND (1)] or [G-^{3}H] indomethacin [^{3}H-IND (2)] gave the corresponding ^{14}C or ^{3}H-labelled indometacin farnesil [^{14}C-IMF (4) or ^{3}H-IMF (5)]. On the otherhand, [3-^{14}C] 6E-3,7,11-trimethyl-2,6,10-dodecatrienol [^{14}C-F (8)] which was obtained from [2-^{14}C] 5E-6,10-dimethylundeca-5,9-dien-2-one [^{14}C-geranyl acetone (7)] in two steps, was esterified with indomethacin to give farnesyl moiety ^{14}C-labelled indometacin farnesil [^{14}C-F-IMF (6)].

Key words; Prodrug of indomethacin, indometacin farnesil
Carbon-14 labelled indometacin farnesil, tritium labelled indometacin farnesil

INTRODUCTION

Indomethacin, a prototype of non steroidal anti-inflammatory drug [1], shows potent anti-inflammatory effects, but its use is restricted because of its side effects such as gastro-intestinal injury [2]. Recently, several types of prodrugs of anti-inflammatory drugs have been developed with the aim of reducing these side effects [3]. E-0710 (IMF), a farnesyl ester of indomethacin, designed along this lines, showed anti-inflammatory activity with diminished gastro-intestinal irritation in several animal models [4),5),6)]. This report describes the synthesis of ^{14}C-labelled IMF and ^{3}H-labelled IMF in order to clear the pharmacokinetic profile of this drug *in vivo* and *in vitro* respectively [7].

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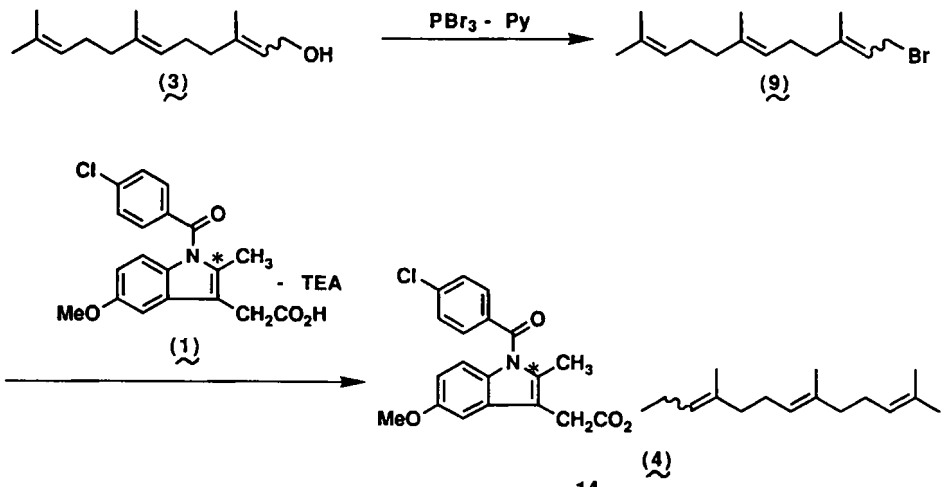
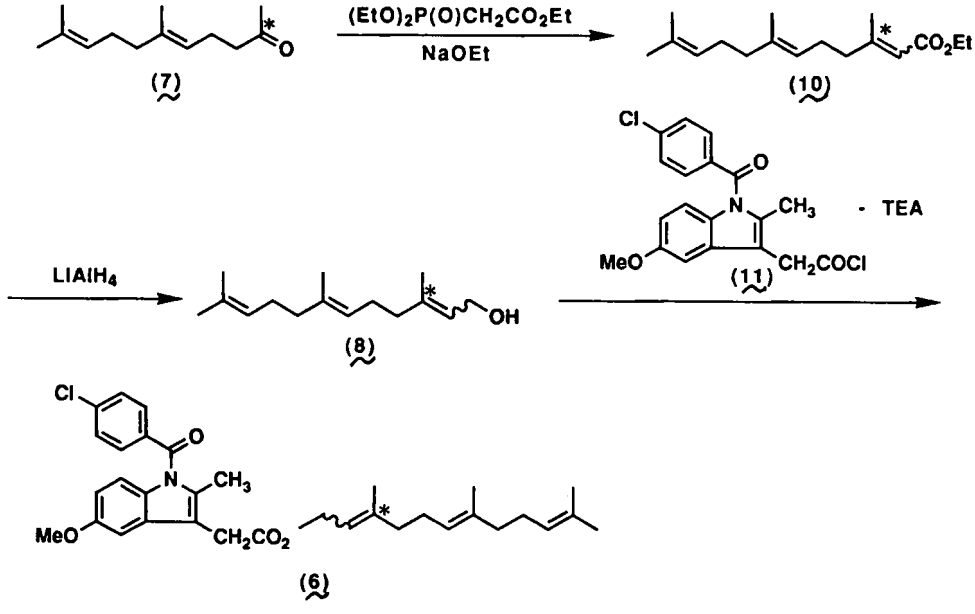


Scheme. 1 Synthesis of ^{14}C-IMF (4)



Scheme. 2 Synthesis of ^{14}C-F-IMF (6)

RESULTS AND DISCUSSION

^{14}C-labelled indomethacin [^{14}C-IND (1)], ^{3}H-labelled indomethacin [^{3}H-IND (2)], and 7:3 (2E:2Z) isomeric mixture of 6E-3,7,11-trimethyl-2,6,10-dodecatrienol [farnesol (3)] are commercially available, so we could prepare ^{14}C-IMF (4) and ^{3}H-IMF (5) only by esterification of (1) and (2) with (3) via its bromide [farnesyl bromide (9)] [8] in the presence of triethylamine as shown in scheme 1. In the case of ^{14}C-F-IMF (6), in which the farnesyl moiety was labelled with ^{14}C, ^{14}C-labelled farnesol [^{14}C-F (8)] must be synthesized at first. [2-^{14}C] 5E-6,10-dimethylundeca-5,9-dien-2-one [^{14}C-geranyl acetone (7)], which could be purchased from Amersham International Ltd., was reacted with triethyl phosphono acetate in the presence of sodium ethoxide to give ethyl [3-^{14}C] 6E-3,7,11-trimethyl-2,6,10-dodecatrienoate (10) [9]. (10) was reduced with lithium aluminium hydride to yield (8). Condensation of (8) with (p-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetyl chloride (11) in the presence of triethylamine gave the desired product (6) as shown in scheme 2. These labelled IMF were purified by column chromatography on silica gel and the structures were confirmed by comparison with unlabelled authentic specimen of IMF on TLC developed by three different solvent systems.

EXPERIMENTAL

Measurements of radioactivity were carried out using an Aloka LSC-9,000 type Liquid Scintillation Spectrometer. Thin layer radiochromatography was performed using a Berthold LB-2,842 Automatic TLC Linear Analyzer. HPLC analysis was carried out with a Waters Model 510 solvent delivery system with a BENSIL 5C18-C analytical column of 4.6mm ID x 15cm. Thin layer chromatography was done using Kiesel 60F plate (MERCK). Wacogel C-200 (Wako chemical Industries) was used for silica gel column chromatography. 7:3 (2E:2Z) isomeric mixture of 6E-3,7,11-trimethyl-2,6,10-dodecatrienol (3) was prepared from Takasago Perfumery Co., Ltd. [2-^{14}C] indomethacin [^{14}C-IND (1)], [G-^{3}H] indomethacin [^{3}H-IND (2)] and [2-^{14}C] 5E-6,10-dimethylundeca-5,9,-dien-2-one [^{14}C-geranyl acetone (7)] were purchased from Amersham International Ltd.

7:3 (2E:2Z) isomeric mixture of 6E-3,7,11-trimethyl-2,6,10-dodecatrienyl [2-^{14}C]-1-(p-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetate [^{14}C-IMF (4)]

Phosphorus tribromide (2.62g; 9.67mmol) was added dropwise with stirring at 0° C to a solution of a 7:3 (2E:2Z) isomeric mixture of

6E-3,7,11-trimethyl-2,6,10-dodecatrienol (3) (3.22g; 14.5mmol) and pyridine (570mg; 7.25mmol) in 100 mL n-hexane and the mixture was stirred at 0° C for an additional 30 min. Ice-water was added to the reaction mixture and the organic layer was separated, washed with brine and dried over magnesium sulfate. The solvent was evaporated under reduced pressure. The resulting residue was dissolved in tetrahydrofuran (10mL) and added to a solution of (1) (546mg; 1.53mmol; 45mCi) and triethylamine (5mL) in tetrahydrofuran (10mL). The reaction mixture was stirred at 30° C for 5 hours and refluxed for 1 hour, then poured into water (20mL) after cooling. Ethyl acetate (20mL) was added to the reaction mixture and the organic layer was separated and washed with brine. The solvent was evaporated under reduced pressure to leave a residue, which was purified by silica gel column chromatography (n-hexane:benzene, 50:50). This yielded, the product (4) (608mg; 70.9% yield from (1)), as a yellow oil, specific activity; 52.4uCi/mg, with a radiochemical purity of greater than 98% as determined by TLC comparison with unlabelled authentic specimen of IMF in the following solvent systems;

a) chloroform:ethyl acetate:acetic acid, 90:10:5 (Rf=0.8)
b) benzene:ethyl acetate, 95:5 (Rf=0.6)
c) benzene:ethyl acetate:methanol:ammonium hydroxide (28%), 50:10:20:0.2 (Rf=0.9)

The ratio of two isomers was determined by HPLC analysis (2E:2Z=65:35) as shown in Fig.1.

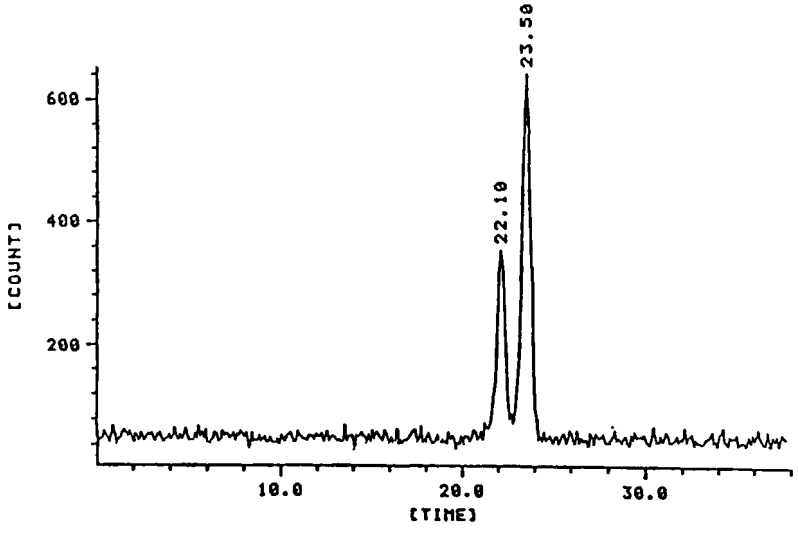


Fig. 1 HPLC Chromatogram of [^{14}C] IMF with Detection by Radioactivity. Column; BENSIL 5C18C 4.6mm ID x 15cm. Elutant; acetonitrile:water(85:15). Flow rate; 2mL/min. Detection; radioactivity.

7:3 (2E:2Z) isomeric mixture of 6E-3,7,11-trimethyl-2,6,10-dodecatrienyl [G-^{3}H] 1-(p-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetate [^{3}H-IMF (5)]

The tritium labelled compound (5) was obtained from [G-^{3}H] indomethacin (2) (877ug; 20mCi) and (3) (30.0mg; 0.135mmol) in the same manner as described above (11.5mCi, 57.5% yield from (2), specific activity; 8.20Ci/mmol, radiochemical purity of greater than 94% as determined by TLC in the solvent systems described above).

[3-^{14}C] 6E-3,7,11-trimethyl-2,6,10-dodecatrienol (8)

Sodium (130mg; 5.65mmol) was dissolved in ethanol (5mL) and the solvent was evaporated. Triethyl phosphonoacetate (1.70g; 7.59mmol) in n-hexane (20mL) was added to the residue and the solution was stirred for 30 min at room temperature. To this mixture, [2-^{14}C] 5E-6,10-dimethyl-5,9-undecadien-2-one (7) (476mg; 2.45mmol; 22mCi) in n-hexane (10mL) was added at room temperature. The reaction mixture was stirred for 1 hour, water-methanol (5mL-5mL) was added and the organic layer was separated. Evaporation of the solvent gave a residue, which was dissolved in ether (15mL) and stirred at 0° C. Lithium aluminium hydride (140mg; 3.70mmol) was added to this solution and the mixture was stirred at 0° C for additional 30 min. After adding water (2.5mL), 1N hydrochloric acid (5mL) and brine (5mL) carefully, the reaction mixture was extracted with n-hexane-ether (30mL-30mL). The organic layer was washed with water (10mL) and evapolated to give a residue, which was purified by silica gel column chromatography (n-hexane:ether, 85:15) to give (8) as a colorless oil (286mg; 11.6mCi). Its structure was confirmed by TLC comparison with unlabelled farnesol (3). (n-hexane:ethyl acetate; 70:30; Rf=0.63 for 2Z isomer and 0.56 for 2E isomer).

[3-^{14}C] 6E-3,7,11-trimethyl-2,6,10-dodecatrienyl-1-(p-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetate [^{14}C-F-IMF (6)]

Indomethacin (1.38g; 3.86mmol) and thionyl chloride (1.38g; 11.6mmol) were dissolved in benzene (10mL) and the solution was refluxed for 30 min. Evaporation of the solvent gave a residue, which was dissolved in tetrahydrofuran (10mL). This acid chloride solution was added to a mixture of (8) (286mg; 1.29mmol) and triethylamine (2mL) in tetrahydrofuran (10mL) at 0° C. The reaction

mixture was stirred for 30 min at room temperature and then poured into 1N-hydrochloric acid (10mL). Ethyl acetate was added, the organic layer was separated, and was then washed with brine. The solvent was evaporated to leave a residue, which was purified by silica gel column chromatography (n-hexane:benzene, 50:50) to give (6) as a yellow oil. (470mg, 64.8% yield from (8), specific activity; 16.0uCi/mg, radiochemical purity of greater than 99% as determined by TLC comparison with unlabelled IMF in the solvent systems described above).

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