LABELLING OF AN ANTI-INFLAMMATORY AGENT WITH CARBON-14, SYNTHESIS OF 5-METHOXY-2-METHYL-1-(3,4-METHYLENEDIOXYBENZOYL)INDOLE-2-^{14}C-3-ACETIC ACID

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SUMMARY

5-Methoxy-2-methyl-1-(3,4-methylenedioxybenzoyl)indole-3-acetic acid (ID-955)(I), a new anti-inflammatory agent, was labelled with carbon-14 at C-2 position of indole nucleus for the use of metabolic studies. The procedure used is shown in Fig. 1 and 2. Levulinic-4-^{14}C acid was synthesized in 57% yield by condensation of ethyl acetoacetate-3-^{14}C with ethyl bromoacetate and subsequent decarboxylation with hydrochloric acid. Reaction of III with N^{1}-(3,4-methylenedioxybenzoyl)-4-methoxyphenylhydrazine (II) gave ID-955-2-^{14}C (I) in 58% yield. A total of 10.6 mCi of pure ID-955-2-^{14}C (I) was obtained, representing 25% radiochemical yield from sodium acetate-1-^{14}C.

Key Words: Indole-3-acetic Acid Derivative, Anti-inflammatory Agent, Levulinic Acid, Carbon-14

INTRODUCTION

In the last decade, since indomethacin[1-4] was found to have an anti-inflammatory activity and has been employed in the treatment of rheumatoid and collagen diseases, a variety of indole-3-acetic acid derivatives has been synthesized and tested as potential anti-inflammatory agents. 5-Methoxy-2-methyl-1-(3,4-methylenedioxybenzoyl)indole-3-acetic acid (ID-955)(I), synthesized in our laboratories[5], exhibits excellent anti-inflammatory, anti-pyretic and analgesic activities. As a matter of course, studies on the metabolic fate of this agent in animals have required the preparation of the carbon-14 labelled

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agent specifically labelled in C-2 position of indole nucleus.

A thorough review of the literature on indole-3-acetic acid derivatives shows the use of indomethacin-2-^{14}C for the metabolic studies[2-4] but no published procedure for the synthesis. The present paper reports a full procedure for the synthesis of ID-955-2-^{14}C.

DISCUSSION

Since the large-scale synthesis of ID-955 devised by Yamamoto, *et al.*[5] comprises allowing levulinic acid to react with N^1-(3,4-methylenedioxybenzoyl)-4-methoxyphenylhydrazine (II) as shown in Fig. 1, the incorporation of carbon-14 into the desired position (C-2) by utilizing this reaction required an effective preparation of levulinic-4-^{14}C acid (III). To our knowledge, however, no

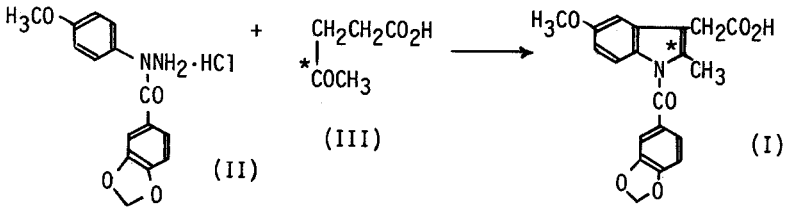

Fig.1. Scheme for the synthesis of ID-955-2-^{14}C (I) from levulinic-4-^{14}C acid (III)

report concerning the synthesis of levulinic-4-^{14}C acid has appeared so far. Although in theory several methods are available to prepare this acid, we selected the procedure illustrated in Fig. 2 because of the relative ease of preparing ethyl acetoacetate-3-^{14}C (V) from sodium acetate-1-^{14}C by the modified method of Dauben[6] and Breslow[7], *et al.*

Acetyl-1-^{14}C chloride[8] which was prepared from sodium acetate-1-^{14}C by means of phosphorus oxychloride was reacted with *t*-butyl ethyl 2-ethoxymagnesiummalonate in refluxing ether for 0.5 hr to give *t*-butyl ethyl 2-acetylmalonate-^{14}C (IV). Without any purification, the acetylmalonate-^{14}C IV was decarboxylated with a catalytic amount of *p*-toluenesulfonic acid to give ethyl acetoacetate-3-^{14}C (V) in 75% of radiochemical yield based on sodium acetate-^{14}C.

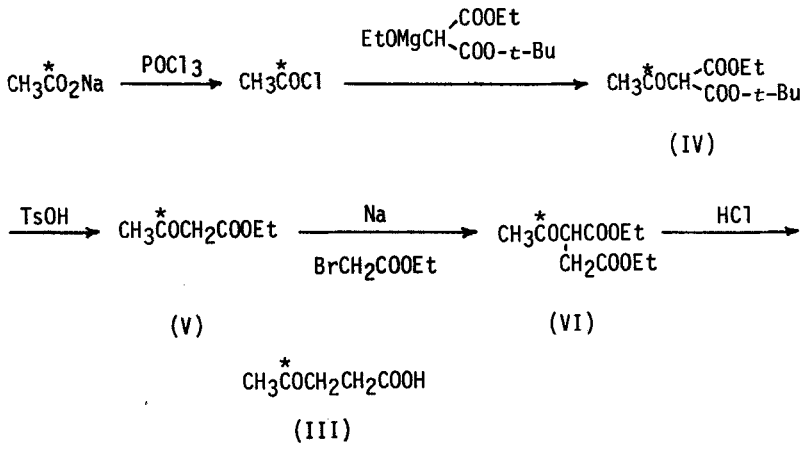

Fig. 2. Scheme for the synthesis of levulinic-4-^{14}C acid (III)

Mentzer, *et al.*[9] reported a large-scale preparation of levulinic acid by basically the same procedure as shown in Fig. 2; including condensation of ethyl acetoacetate with ethyl chloroacetate in the presence of sodium ethoxide and subsequent decarboxylation of the resulting succinate VI with 17% hydrochloric acid. Contrary to the reported requirements, we found that the best yield (57%) of levulinic-4-^{14}C acid (III) was obtained by allowing ethyl acetoacetate-3-^{14}C (V) to react with sodium metal in toluene and subsequently with ethyl bromoacetate at the higher reaction temperature (90°) followed by decarboxylation with 7% hydrochloric acid. The product obtained was of sufficient high purity (95%) to allow its use in the following reaction.

The method of Yamamoto[5] to prepare ID-955 (I) in which a very excess of levulinic acid is used to the phenylhydrazine II is not readily applicable to this isotopic preparation. After considerable investigation, a condition was established under which reasonable yields of I could be obtained. Thus, levulinic-4-^{14}C acid in acetic acid was allowed to react with 15% excess of the phenylhydrazine II at 75-80° for 3 hr to afford a crude product which was purified by column chromatography on alumina and subsequent recrystallization from ethanol; giving pure ID-955-2-^{14}C (I) in 58% yield. The overall yield of I based on sodium acetate-1-^{14}C was nearly 25%.

EXPERIMENTAL

Acetyl-1-^{14}C Chloride -- Acetyl-1-^{14}C chloride was prepared from sodium acetate-1-^{14}C (57.2 mCi, 1.01 g, 12.3 mmoles) according to Olsen's method[8]; in which sodium acetate-1-^{14}C was allowed to react with phosphorus oxychloride (3.0 g, 20 mmoles) at room temperature for 24 hr. The resulting product was distilled in a vacuum manifold into a receiver to give acetyl-1-^{14}C chloride, which was used in the following reaction with further purification by redistillation.

Ethyl Acetoacetate-3-^{14}C (V) -- To magnesium turnings (340 mg, 14 mmoles) were added absolute ethanol (0.7 ml) and carbon tetrachloride (0.05 ml) at room temperature. After a few minutes a vigorous reaction started. The flask was cooled and anhydrous ether (7 ml) was added. To the mixture was added dropwise *t*-butyl ethyl malonate (3.01 g, 16 mmoles) in absolute ethanol (1 ml), the reaction mixture refluxing during the addition. After complete addition, the mixture was refluxed for 6 hr. After cooling, the reaction flask was connected to a vacuum manifold and the mixture was frozen with liquid nitrogen. To the frozen mixture was added under reduced pressure, by distillation, the acetyl-1-^{14}C chloride obtained above. The mixture was then refluxed under atomospheric pressure for 0.5 hr, cooled, diluted with water (20 ml) and acidified with 1N hydrochloric acid (5 ml). The aqueous solution was separated and extracted with ether. The extract was dried over sodium sulfate and evaporated to give an oily residue of crude *t*-butyl ethyl 2-(acetyl-1-^{14}C)malonate.

A mixture of the oily residue and *p*-toluenesulfonic acid (150 mg) in anhydrous benzene (20 ml) was refluxed for 1.5 hr. The benzene solution was cooled, washed with 5% sodium bicarbonate solution and then saturated sodium chloride solution, and dried over sodium sulfate. Evaporation of the solvent gave ethyl acetoacetate-3-^{14}C (V)(43.1 mCi, 1.24 g, 75.4% radiochemical yield from sodium acetate-1-^{14}C); the radiochemical purity was shown to be about 93% by radiogaschromatography (column: DC-550, 1 m; temperature: 120°; flow rate: 120 ml/min; retention time: 10 min) and its IR-spectrum was identical with that of the unlabelled authentic sample.

Levulinic-4-^{14}C Acid (III) -- To a solution of ethyl acetoacetate-3-^{14}C (43.1 mCi, 1.56 g, 12 mmoles) in anhydrous toluene (25 ml) sodium metal (304 mg, 13.2 mmoles) was added gradually with stirring, and then the mixture was heated at 60° for 3 hr. To the mixture ethyl bromoacetate (2.20 g, 13.2 mmoles) was added portionwise at 60° and the mixture was heated at 90° for 4 hr. After cooling, the inorganic precipitate which had formed was filtered and the solid was washed with benzene. The combined filtrate was evaporated to give an oily residue of diethyl 2-(acetyl-1-^{14}C)succinate (VI). The residue was heated with 7% hydrochloric acid (50 ml) at 90° for 4 hr. The mixture was extracted continuously with ether for 24 hr. The ethereal extract was dried over sodium sulfate and evaporated to give levulinic-4-^{14}C acid (III)(24.6 mCi, 796 mg, 57% from V). Its purity was shown to be 95% by radio-thinlayerchromatography on silica gel with chloroform-acetone-formic acid (90/10/1 v/v)(R_f=0.28), and its IR-spectrum (liquid film) showed a very strong and broad absorption at 1720 cm^{-1} (acetyl and acid C=O) and was identical in every respect with that of the unlabelled authentic sample. The product was used in the following reaction without any purification.

5-Methoxy-2-methyl-1-(3,4-methylenedioxybenzoyl)indole-2-^{14}C-3-acetic Acid (ID-955-2-^{14}C)(I) -- Levulinic-4-^{14}C acid (18.3 mCi, 596 mg, 5.14 mmoles) and unlabelled levulinic acid (116 mg, 1 mmole) were dissolved in acetic acid (3 ml). To the solution was added portionwise N^1-(3,4-methylenedioxybenzoyl)-4-methoxyphenylhydrazine hydrochloride (2.25 g, 7.0 mmoles) at 75-80°, and the mixture was kept at this temperature for 3 hr. After cooling, the mixture was diluted with water (15 ml) and extracted with ethyl acetate, and the extract was washed with water, dried over sodium sulfate and evaporated to afford an oily residue. The residue was chromatographed on alumina and eluted with acetone and acetone-water (9/1 v/v) successively. The second eluate (410 ml) was concentrated to approximately 200 ml and acidified with 10% hydrochloric acid to produce a pale yellow precipitate, which was collected by filtration and recrystallized from ethanol to give 5-methoxy-2-methyl-1-(3,4-methylenedioxybenzoyl)indole-2-^{14}C-3-acetic acid (ID-955-2-^{14}C)(10.6 mCi, 1.70 g, 58% yield from III) as pale

yellow needles; mp and mixed mp 160-162°, specific activity of 2.27 mCi/mmole, identical in every respect with the unlabelled authentic sample.

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