SYNTHESIS OF trans-[13,14-$^{14}C_2$]-RETINOIC ACID

Heinz Kaegi,* Edward Chew, and Ping-Lu Chien**
Midwest Research Institute
425 Volker Boulevard
Kansas City, MO 64110 USA

SUMMARY

trans-[13,14-$^{14}C_2$]-Retinoic acid has been synthesized by condensation of β-ionylideneacetaldehyde and ^{14}C-labeled methyl senecioate in the presence of potassium amide in ether. A convenient synthesis of the key intermediate, methyl (diethoxyphosphinyl)-[2-^{14}C]acetate, is also described.

Key Words: trans-[13,14-$^{14}C_2$]-Retinoic Acid, Methyl [2,3-$^{14}C_2$]-3-methyl-2-butenoate, Methyl (diethoxyphosphinyl)-[2-^{14}C]acetate

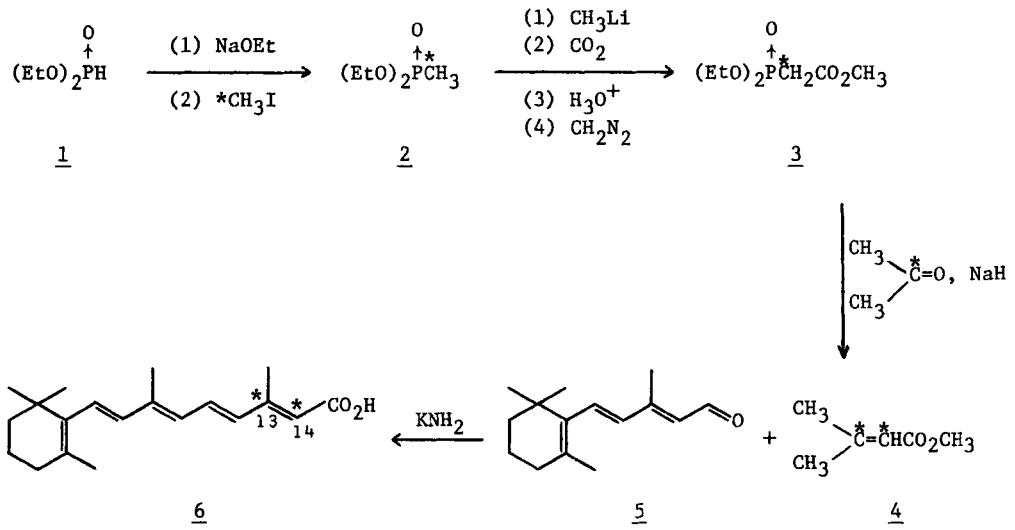
Condensation of β-ionylideneacetaldehyde (5) with a 3-methyl-2-butenoate ester in liquid ammonia or anhydrous ether in the presence of an alkali metal amide, according to the procedure described by Matsui and co-workers [1], is one of the most convenient synthetic routes to retinoic acids and their derivatives. This reaction appears to be particularly valuable in the synthesis of labeled retinoic acids [2] needed for metabolic and biological studies [3], since only one of the two isomers would be formed preferentially depending on the individual amide used. Thus, when potassium amide is employed as the base for the condensation, the product is predominantly trans-retinoic acid, whereas the 13-cis isomer is the major product with sodium or lithium amide as the basic metalating agent for the senecioate [4]. This condensation reaction has now been successfully utilized by us to synthesize the double ^{14}C-labeled retinoic acid, trans-[13,14-$^{14}C_2$]-retinoic acid (6), from β-ionylideneacetaldehyde (5) and methyl [2,3-$^{14}C_2$]-3-methyl-2-butenoate 4.

* Present address: SRI International, Menlo Park, California 94025.
** To whom correspondence regarding this paper should be addressed.

0362-4803/80/0517-0745$01.00

Received May 25, 1979
Revised June 29, 1979

Scheme I



Methyl (diethoxyphosphinyl)-[2-^{14}C]acetate (3), the intermediate needed in the synthesis of the labeled methyl senecioate 4, was prepared directly from [^{14}C]iodomethane following a novel method recently reported by Coutrot, et al. [5], as shown in Scheme I. By using this procedure, the necessity of converting [^{14}C]iodomethane to methyl [2-^{14}C]bromoacetate and then subjecting it to the Arbuzow reaction to form the phosphonoacetate 3 was circumvented. The methylation of diethyl phosphite (1) was carried out readily by treatment of its anion with [^{14}C]iodomethane. The [2-^{14}C]methylphosphonate 2 thus obtained was lithiated with methyllithium, and subsequent carbonation, neutralization, and esterification furnished methyl (diethoxyphosphinyl)-[2-^{14}C]acetate (3) in an overall yield of 55%.

The phosphonacetate 3 readily underwent Wittig-Horner reaction with [2-^{14}C]acetone to give a satisfactory yield of the double labeled methyl 3-methyl-2-butenoate 4. To minimize decomposition due to radiolysis, this intermediate was distilled quickly in vacuo at low temperature and then used immediately for

reaction with β-ionylideneacetaldehyde. As usual, the Matsui condensation was carried out in anhydrous ether [6] in the presence of potassium amide under similar conditions as those used in pilot "cold" runs. The crude product formed in this reaction (76% yield) was shown by reverse phase HPLC analysis [7] to contain both the desired trans-[13,14-$^{14}C_2$]-retinoic acid (6) and its 13-cis isomer roughly in the ratio of 2:1. One rapid crystallization of the crude retinoic acid mixture from methanol then furnished the labeled trans-retinoic acid with a radiochemical purity of 95%. The specific activity of the product was 51 mCi/mmole.

EXPERIMENTAL

[^{14}C]Iodomethane used in this work was purchased from Amersham Corporation. [2-^{14}C]Acetone was prepared in 57% yeild by treatment of [1-^{14}C]acetyl chloride with dimethylcadmium (Ventron) [8]. Reverse phase HPLC analysis was carried out on a Spectro-Physics Model 3500 liquid chromatography unit equipped with Waters Associates Model 440 dual wavelength detector, using a Whatman Partisil PXS 10/25 ODS-2 column (solvent composition: acetonitrile vs. 1% aq. acetic acid, 35% to 5% aq. at 3% per minute; flow rate 1.1 ml/min; elution time for 13-cis- and trans-retinoic acid: 14 and 17 min, respectively). Radioactivity was measured by liquid scintillation counting using a Beckman LS-100 scintillation counter. Radiochemical purity was determined by plotting the radioactivity found in each of 200 fractions (∿ 0.15 ml) collected during HPLC analysis vs. number of fractions. GC analysis was performed on a Varian Aerograph Series 2400 instrument equipped with a Carbowax 20M PTA column.

Diethyl [^{14}C]Methylphosphonate (2)

A solution of 1.15 g (8.33 mmol) of redistilled diethyl phosphite in 5 ml of benzene was added slowly, with stirring, to a sodium ethoxide solution

prepared from 0.21 g (9 mg-atoms) of sodium and 7 ml of anhydrous ethanol under argon. After stirring for 1 hr at room temperature, the mixture was cooled in liquid nitrogen and evacuated. Thereupon, [^{14}C]iodomethane (8.33 mmol, 250 mCi) was added *via* vacuum transfer. The cooling bath was removed and the reaction mixture was stirred overnight at room temperature. At this point, an additional 2.4 mmol of unlabeled iodomethane was introduced and stirring was continued for 1.5 hr. Lower boling materials were removed from the resulting reaction mixture *via* vacuum transfer, and the residue was treated with 5 ml of water and extracted with a 1:1 mixture of dichloromethane and ethyl acetate (total 80 ml). The extract was dried ($MgSO_4$) and evaporated to give 1.12 g (200 mCi, specific activity: 27 mCi/mmol) of diethyl [^{14}C]methylphosphonate as an oily liquid. The product was characterized by co-chromatography (TLC) with an unlabeled standard. The radiochemical yield was 80%.

Methyl (Diethoxyphosphinyl)-[2-^{14}C]acetate (3)

To a solution of 653 mg (4.3 mmol, 116 mCi) of diethyl [^{14}C]methylphosphonate in 5 ml of anhydrous THF was added 5 ml of 2 M methyllithium-lithium bromide complex (Aldrich) *via* a syringe at -65°. After the mixture was stirred at -65° for 1 hr, 15 mmol of dry carbon dioxide was introduced by vacuum transfer and stirring was continued for 2.5 hr. The reaction mixture was then allowed to warm up slowly to 10°. Water (5 ml) was added and the solvents evaporated under reduced pressure. The residue was acidified with dilute sulfuric acid to pH 2 and extracted exhaustively with a 3:2 mixture of ether and dichloromethane (total 180 ml). The organic solution was dried ($MgSO_4$) and evaporated *in vacuo* to give 110 mCi of (diethoxyphosphinyl)-[2-^{14}C]acetic acid as a clear oily liquid. The crude product was dissolved in ether and treated with a large excess of diazomethane. The resultant methyl ester was purified by preparative TLC on Whatman Quanta-gram PKSF plates (1:2 hexane-acetone as eluent), affording 650 mg (3.1 mmol, 80 mCi) of

the desired methyl ester of (diethoxyphosphinyl)-[2-^{14}]acetic acid (69% yield).

Methyl [2,3-$^{14}C_2$]-3-Methyl-2-butenoate (4)

A solution of 3.1 mmol (80 mCi) of methyl (diethoxyphosphinyl)-[2-^{14}C]-acetate in 10 ml of ether was added slowly to 158 mg (3.3 mmol) of 50% sodium hydride in oil at -10° under argon. After stirring 1 hr at room temperature, the mixture was cooled in liquid nitrogen and evacuated. By vacuum transfer, 2.8 mmol (80 mCi) of [2-^{14}C]acetone was added. The resultant mixture was stirred at room temperature for 3.5 hr before an additional 2 mmol of unlabeled acetone was introduced. Stirring was continued overnight. On fractional distillation of the resulting reaction mixture, the desired methyl [2,3-$^{14}C_2$]-3-methyl-2-butenoate was obtained as a clear liquid. The total activity in the product was 150 mCi, and it was shown by GC to be approximately 80% pure. The crude product was immediately used in the following reaction without further purification.

trans-[13,14-$^{14}C_2$]-Retinoic Acid (6)

To 25 ml of redistilled anhydrous ammonia was added, under argon, 0.34 g of potassium followed by a few crystals of ferric nitrate hydrate. The mixture was stirred under reflux for 30 min, cooled in a dry-acetone bath and treated with 10 ml of ether and 300 µl of absolute ethanol. The cooling bath was removed and ammonia was allowed to evaporate. The resultant solid suspension in ether was cooled again to -65° and a mixture of the crude methyl [2,3-$^{14}C_2$]-3-methyl-2-butenoate obtained in the preceding experiment and 478 mg (2.2 mmol) of β-ionylideneacetaldehyde [9] in 15 ml of ether was added *via* a syringe through a rubber septum. The raction mixture was stirred at -65° for 1 hr and then at room temperature for 17 hr. Methanol (3 ml) was added, followed by 25 ml of water. After an additional 1 hr of stirring the aqueous layer was withdrawn and the ether solution

was washed with 15 ml of dilute sodium hydroxide. The combined aqueous solution was made acidic to pH 3 with 3 N phosphoric acid and the yellow crystals which formed were taken up in ether (120 ml). The ether solution was washed with water (3X), dried ($MgSO_4$), and evaporated to give 0.50 g (76%) of crude retinoic acid mixture containing the $^{14}C_2$-labeled 13-cis- and trans-retinoic acid in the ratio of 1:2 as analyzed by reverse phase HPLC. On recrystallization from methanol there was obtained 164 mg of the desired trans-[13,14-$^{14}C_2$]-retinoic acid as yellow crystals with a specific activity of 51 mCi/mmol. The radiochemical purity was 95% as determined by HPLC analysis.

ACKNOWLEDGMENT

This work is supported by the Lung Cancer Branch, National Cancer Institute, under Contract No. NO1-CP-75911. The authors wish to thank Dr. William P. Duncan for helpful discussion and Mr. Patrick Crawford for HPLC analysis.

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