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SYNTHESIS OF [^{14}C]-Labelled AY-30,068

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SUMMARY

[^{14}C]AY-30,068 (cis-1,8-diethyl-2,3,4,9-tetrahydro-4-(2-propenyl)-1H-carbazole-1-acetic acid), a potent analgesic agent, was prepared by incorporating [^{14}C]methyl iodide via a Wittig reaction. The intermediate aldehyde was synthesized in six steps from cis-1-ethyl-2-oxo-4-(2-propenyl)cyclohexaneacetic acid methyl ester. Three batches of the [^{14}C]labelled AY-30,068 were produced, giving a combined overall yield of 9% from [^{14}C]methyl iodide (sp. act. 51.2, 17.7 and 4.4 μCi/mg; 97.5, 98.3, and 98.6% radiochemcical purity, respectively).

Key words: AY-30,068, analgesic, ^{14}C, Wittig reaction.

INTRODUCTION

AY-30,068 (I) is an allyl substituted carbazole derivative that exhibits potent analgesic activity (1). In order to study the metabolic disposition of AY-30,068 in laboratory animals, a synthesis of the [^{14}C]labelled compound was undertaken as shown below:

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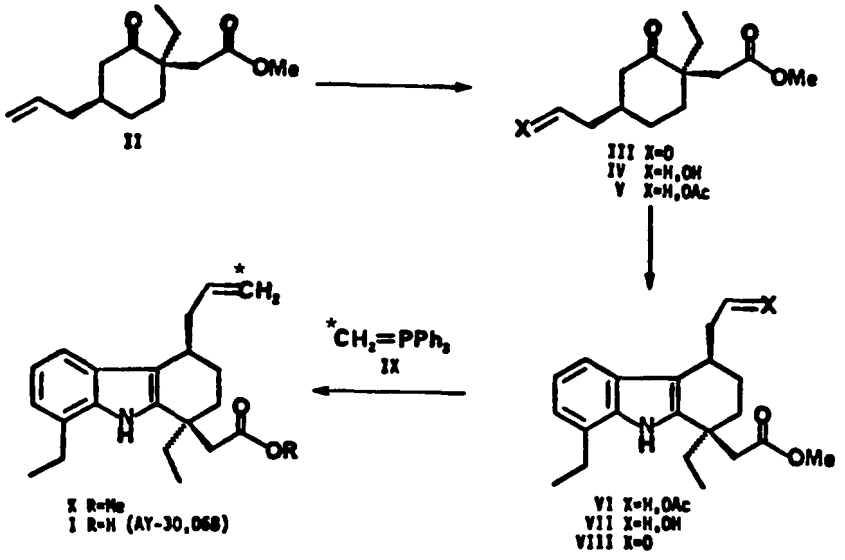

*Position of ^{14}C label

DISCUSSION

The key intermediate in the synthesis of [^{14}C]AY-30,068 was the aldehyde VIII. The preparation of this compound required the prior synthesis of cis-1-ethyl-2-oxo-4-(2-propenyl)cyclohexaneacetic acid methyl ester (II) (2). The aldehyde III, obtained when II was treated with sodium periodate and catalytic osmium tetroxide, was reduced with lithium tris[(3-ethyl-3-pentyl)oxy]aluminum hydride (3). The resulting primary alcohol IV was then acetylated to give V. Fisher indole cyclization was accomplished by heating V in toluene with 2-ethylphenylhydrazine followed by treatment of the resulting hydrazone with boron trifluoride etherate in acetic acid. Hydrolysis of the acetate VI followed by oxidation of VII with Dess-Martin periodinane (4) gave the required aldehyde VIII.

The [^{14}C]*methyl triphenylphosphonium iodide*, obtained when triphenylphosphine and [^{14}C]methyl iodide were mixed together, was converted to the ylide IX on treatment with methyllithium (5,6). The [^{14}C]labelled ylide was condensed with the aldehyde VIII and the resulting ester X was hydrolyzed to [^{14}C]AY-30,068. The [^{14}C]AY-30,068 was isolated in three batches and was obtained in an overall radiochemical yield of 9% based on [^{14}C]methyl iodide: 0.0111

g, sp. act. 51.2 μCi/mg; 0.0703 g, sp. act. 17.7 μCi/mg; and 0.4360 g, sp. act. 4.4 μCi/mg. The radiochemical purity was determined to be 97.5, 98.3 and 98.6%, respectively, by TLC autoradiography in three solvent systems.

EXPERIMENTAL

The synthesis of [^{14}C]AY-30,068 was carried out using [^{14}C]methyl iodide (35 mCi, sp. act. 57 mCi/mmole) purchased from Amersham Corp., Arlington Height, Ill. The intermediates in the synthesis were characterized in trial experiments with unlabelled material. The reactions in the labelled synthesis were monitored by TLC using unlabelled reference compounds.

Cis-1-Ethyl-2-oxo-4-(2-oxoethyl)cyclohexaneacetic Acid Methyl Ester (III)

A solution of II (42.02 mmole, 10 g) in THF (282 ml) and water (93 ml) was stirred mechanically at 0° and treated with OsO_4 (214 mg, 0.840 mmole, 10.83 ml of 2.5% w/w solution in tert-butanol, d = 0.7887). After 15 min, the brown reaction mixture was treated with sodium periodate (126.1 mmole, 26.96 g), the ice bath was removed and the off-white viscous reaction was stirred for 1 hr. It was diluted with brine (300 ml) and extracted with ether (4 x 200 ml). Drying ($MgSO_4$) and flash chromatography (95 mm column, 35% EtOAc/pet ether eluant) afforded a dark brown oil (6.89 g), ^{1}H NMR ($CDCL_3$/TMS, 400 MHz): δ 0.81 (t, 3H, J=7.62, $CH_2\underline{CH_3}$), 1.5-2.8 (m, 13H, ring $\underline{CH_2}$, $CH_1\underline{CH_2}CH_3$, $\underline{CH_2}CHO$), 3.65 (s, 3H, $CO_2\underline{CH_3}$), 9.78 (s, 1H, $\underline{CHO}$); IR (neat) 3000-2800 (CH), 2710 (CHO), 1740-1690 (C=O) cm^{-1}.

Cis-1-Ethyl-4-(2-hydroxyethyl)-2-oxocyclohexaneacetic Acid Methyl Ester (IV)

Lithium aluminum hydride (200 mmole, 7.59 g) was stirred in tetrahydrofuran (800 ml) at room temperature under nitrogen and treated dropwise with 3-ethyl-3-pentanol (620 mmole, 72.04 g, 87.43 ml = 90.1 ml of 97%). After completion of the addition, the reaction mixture was refluxed for 1 hour. It was then transferred to a graduated addition funnel where the volume was measured to be 950 ml, thus affording a 0.21 M solution of the reducing agent. Of this, 132 ml were added dropwise to a solution of III (124 mmole, 29.8 g) in tetrahydrofuran (496 ml) at

-78°C under nitrogen. After 25 min, the reaction was quenched gradually with portions of 1M HCl (total volume added = 530 ml) as it was warmed to room temperature. The mixture was extracted with ether (1x400 ml and 2x200 ml) and the extracts were combined, dried over magnesium sulfate and concentrated. The remaining 3-ethyl-3-pentanol was removed by distillation under full vacuum (b.p. = room temperature) leaving the primary alcohol (brown oil, 27.9 g), in the pot: ^{1}H NMR ($CDCl_3$/TMS, 200 MHz) δ 0.8 (t, 3H, J=7.8, $CH_2\underline{CH_3}$), 1.5-2.5 (m, 11H, ring $\underline{CH_2}$, $\underline{CH}$, $\underline{CH_2}CH_3$, $\underline{CH_2}CH_2OH$), 2.5 (d, 1H, J=15, $\underline{CH_2}CO_2$), 2.6 (d, 1H, J=15, $\underline{CH_2}CO_2$), 3.6 (s, 3H, $CH_2\underline{CH_3}$), 3.7 (t, 2H, J=6.81, $CH_2\underline{CH_2}OH$); IR (neat) 3410 (OH), 2920 (CH), 2860 (CH), 1720 (C=O) cm^{-1}.

Cis-1-Ethyl-4-(2-acetoxyethyl)-2-oxocyclohexaneacetic Acid Methyl Ester (V)

A solution of crude IV (27.0 g, 115 mmole) in dry methylene chloride (115 ml) was treated with triethylamine (172 mmole, 17.5 g, 24.1 ml) followed by acetic anhydride (150 mmole, 15.3 g, 14.6 ml) and finally 4-dimethylaminopyridine (a few mg). The reaction was stirred for 20 min and then diluted with n-pentane (150 ml) and washed with 1M HCl (2 x 100 ml) and saturated aqueous $NaHCO_3$ (2 x 100 ml). Drying ($MgSO_4$) and flash chromatography (95 mm column, 15% ethyl acetate in petroleum ether eluant) afforded a yellow oily product (29.7 g), ^{1}H NMR ($CDCl_3$/TMS, 200 MHz) δ 0.8 (t, 3H, J=7.6, $CH_2\underline{CH_3}$), 1.4-2.6 (m, 13H, ring $\underline{CH_2}$, $\underline{CH}$, $\underline{CH_2}CH_2O$, $\underline{CH_2}CO_2$), 2.0 (s, 3H, $O_2C\underline{CH_3}$), 3.6 (s, 3H, $CO_2\underline{CH_3}$), 4.1 (t, 2H, J=6.5, $CH_2\underline{CH_2}CO_2$); IR (neat) 3000-2850 (CH), 1730 (C=O), 1705 (C=O) cm^{-1}.

Cis-1,8-Diethyl-2,3,4,9-tetrahydro-4-(2-hydroxyethyl)-1H-carbazole -1-acetic Acid Methyl Ester (VII)

A solution of V (35.2 mmole, 10 g) and 2-ethylphenylhydrazine (38.7 mmole, 5.28 g) in toluene (100 ml) was refluxed for 4 h under nitrogen with azeotropic removal of water. At this point, TLC analysis demonstrated complete formation of the hydrazone and the reaction was stored in a freezer overnight. The toluene was then removed on a rotavap and replaced with acetic acid (25 ml). This solution was treated with boron trifluoride etherate (45.8 mmole, 6.50 g, 5.6 ml) and refluxed under nitrogen for 20 min. The reaction mixture was then poured into water (250 ml) and extracted with ether (4 x 100 ml). The

extracts were combined and washed with 1M HCl followed by 2.5M NaOH (2 x 100 ml). Drying ($MgSO_4$) and flash chromatography (95 mm column, 15% ethyl acetate in petroleum ether eluant) afforded oily VI (6.52 g, 16.9 mmole, 48%). Crude VI (3.20 g, 8.31 mmole) was stirred under nitrogen in methanol (17 ml) and treated with anhydrous potassium carbonate (230 mg, 1.66 mmole). After 1.33 h, the reaction was quenched with 1M HCl (1.6 ml), stripped of solvents and redissolved in ether (40 ml). It was washed with brine (20 ml) dried over $MgSO_4$ and concentrated to afford an orange oil (2.89 g). Flash chromatography (50 mm column, 40% ether/petroleum ether eluant) afforded a yellow-orange oil (2.12 g), ^{1}H NMR ($CDCl_3$/TMS, 200 MHz) δ 0.85 (t, 3H, J=7.4, $CH_2\underline{CH_3}$), 1.38 (t, 3H, J = 7.4, $ArCH_2\underline{CH_3}$), 1.6-2.4 (m, 8H, ring $\underline{CH_2}$,$\underline{CH_2}CH_3$, $\underline{CH_2}CH_2OH$), 2.6 (d, 1H, J=16, $\underline{CH_2}CO_2$), 2.7 (d, 1H, J=16, $\underline{CH_2}CO_2$), 2.9 (q, 2H, J=7.6, $Ar\underline{CH_2}CH_3$), 3.2 (m 1H, ring CH), 3.72 (S, 3H, $CO_2\underline{CH_3}$), 3.83 (t, 2H, J=6.7, $CH_2\underline{CH_2}OH$), 7.3 (m, 2H, aromatic), 7.43 (d of d, 1H, J=1.8 and 6.9, aromatic), 9.4 (broad s, 1H, NH); IR (thin flim) 3460 (NH), 3030 (aromatic CH), 3000-2860 (aliphatic CH), 1700 (C=O) cm^{-1}.

Cis-1,8-Diethyl-4-(2-oxoethyl)-2,3,4,9-tetrahydro-1H-carbazole-1-acetic Acid Methyl Ester (VIII).

A solution of Dess-Martin Periodinane (0.33 mmole, 140 mg) in methylene chloride (1.34 ml, distilled from CaH_2) was stirred at 0° under nitrogen and treated dropwise with a solution of VII (0.30 mmole, 103 mg) in methylene chloride (1.08 ml). After 10 min the reaction was diluted with ether (5 ml) and poured into a saturated aqueous solution of sodium bicarbonate (8 ml) containing sodium thiosulfate (2.31 mmole, 365 mg). The layers were separated and the aqueous layer was extracted with ether (4 x 5 ml). The extracts were combined, dried over Mg SO_4 and concentrated. Flash chromatography (20 mm column, 25% ethyl acetate in petroleum ether eluant) afforded an oily product (79 mg), ^{1}H NMR ($CDCl_3$/TMS, 200 MHz) δ 0.86 (t, 3H, J=7.4, $CH_2\underline{CH_3}$), 1.38 (t, 3H, J=7.7, $ArCH_2\underline{CH_3}$), 1.4-2.0 (m, 6H, ring CH_2, $\underline{CH_2}CH_3$), 2.4-2.8 (m, 6H, $\underline{CH_2}CO_2$, $Ar\underline{CH_2}CH_3$, $\underline{CH_2}CHO$), 3.6 (m, 1H, ring CH), 3.73 (S, 3H, $CO_2\underline{CH_3}$), 7.0 (m, 2H, aromatic), 7.3 (m, 1H, aromatic), 9.6 (broad s, 1H, NH), 9.9 (m, 1H, $\underline{CH}O$); IR (neat) 3380 (NH), 3000-2880 (aliphatic CH), 2720 ($\underline{CH}O$), 1720 (CHO) cm^{-1}.

[^{14}C]Methylenetriphenylphosphorane (IX)

An apparatus was constructed consisting of a 25 ml reaction tube

connected to a breakseal ampule via a distillation adaptor containing the labelled methyl iodide (35 mCi, 57 mCi/mmole, 0.6 mmole). The distillation adaptor was filled with dry soda lime to trap any iodine present in the methyl iodide. The reaction tube was charged with triphenylphosphine (177.2 mg, 0.68 mmole) and dry tetrahydrofuran (3.0 ml) and frozen in a liquid nitrogen bath. The apparatus was connected to a vacuum pump and evacuated to 0.1 mm Hg, filled with nitrogen and evacuated again. The apparatus was isolated from the vacuum pump, and the seal on the breakseal ampule was broken. After the contents of the ampule had evaporated, the ampule and distillation adaptor were gently warmed to complete the transfer of methyl iodide. Nitrogen was let into the system, and the contents of the reaction tube were warmed slowly to room temperature. A fine white precipitate began forming shortly thereafter.

After stirring overnight, the resulting phosphorane was cooled to 0° and treated with methyllithium (1.2M, 708 µl, 0.84 mmole). The resulting yellow suspension was stirred at 0° for 15 min, warmed to room temperature, and stirred for an additional 15 min. The resulting phosphorous ylide was used in the next step of the synthesis.

Cis-4-([3-^{14}C]-2-propenyl)-1,8-diethyl-2,3,4,9-tetrahydro-1-H-carbazole-1-acetic acid (AY-30,068) (I).

The phosphorous ylide was cooled to 0° and treated with a solution of the aldehyde VIII (194 mg, 0.57 mmole) in dry tetrahydrofuran (3.0 ml). The reaction was stirred for 5 min at which time TLC (20% ethyl acetate/hexane) indicated that no aldehyde remained. The mixture was diluted with methanol (10.0 ml), transferred to a 50 ml flask, treated with 1N NaOH (2.5 ml), and heated to reflux for 4.5 hr. The solution was cooled to room temperature, poured into water (50 ml), acidified with 1N HCl to pH 1, and extracted with ether (3 x 25 ml). The combined ether extracts were washed with water (2 x 50 ml), dried over magnesium sulfate, and evaporated to dryness under vacuum to yield crude [^{14}C]AY-30,068 (372 mg).

The crude [^{14}C]AY-30,068 was mixed with unlabelled AY-30,068 (110 mg) and crystallized from benzene/petroleum ether. The crystals (14.7 mg) were collected, mixed with unlabelled AY-30,068 (3.5 mg), and crystallized from benzene/petroleum ether. The [^{14}C]AY-30,068 was collected and dried under vacuum at 65° (11.1 mg; sp. act. 51.2 µCi/mg).

The mother liquors from the first batch of [^{14}C]AY-30,068 were mixed with unlabelled AY-30,068 (293 mg) and crystallized from benzene/petroleum ether (70.3 mg, sp. act. 17.7 μCi/mg). In a similar fashion, a third batch of labelled AY-30,068 was obtained from the mother liquors of the second batch (436 mg, sp. act. 4.4 μCi/mg).

The radiochemical purity of each batch was determined by TLC autoradiography using three solvent systems: a) $CHCl_3$: Hexane: HOAC = 20:5:0.1; b) Hexane: EtOAc: HOAc = 18:7:0.1; and c) $CHCl_3$: EtOAc: HOAc = 43:7:0.2 and 1% H_3PO_4 dipped TLC plates. The radioactive zones were located by exposing the plates to Kodak XAR-5 X-ray film. The silica gel (1 cm sections) was scraped into scintillation vials, digested with water (0.2 ml) and 50% hydrofluoric acid (0.2 ml), and counted in Aquasol (15.0 ml).

The TLC, IR, and NMR properties of the [^{14}C]AY-30,068 were identical to those of an authentic sample of AY-30,068: ^{1}H NMR ($CDCl_3$/TMS, 60 MHz) δ 0.85 (t, 3H, J=8, $CH_2\underline{CH_3}$), 1.33 (t, 3H, J=8, $ArCH_2\underline{CH_3}$), 1.6-2.5 (m, 6H, ring $\underline{CH_2}$, $\underline{CH_2}CH_3$), 2.5-3.4 (m, 7H, ring $\underline{CH}$, $\underline{CH_2}CO_2$, $ArCH_2\underline{CH_3}$, $\underline{CH_2}CH=$), 4.9-5.3 (m, 2H, $=\underline{CH_2}$), 5.6-6.3 (m, 1H, $\underline{CH}=$), 7.0-7.6 (m, 3H, aromatic), 8.98 (broad s, 1H, NH); IR (KBr) 3600-3100 (CO_2H), 3400 (NH), 1710 (C=O) cm^{-1}.

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