

SYNTHESIS OF [4-¹⁴C]DIOSGENIN

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SUMMARY

[4-¹⁴C]-(25R)-Spirost-5-en-3-ol(diosgenin), specific activity 3.77 ± 0.02 mCi/mmole, was synthesized in four steps from (25R)-5-hydroxyspirost-5-en-3-*oic*-3,5-lactone **4** and [¹⁴C]methyl magnesium iodide with an overall yield of 18%. The preparation of the starting lactone from diosgenin is also described.

Key Words: Diosgenin, (25R)spirostane, Carbon-14, 4-position

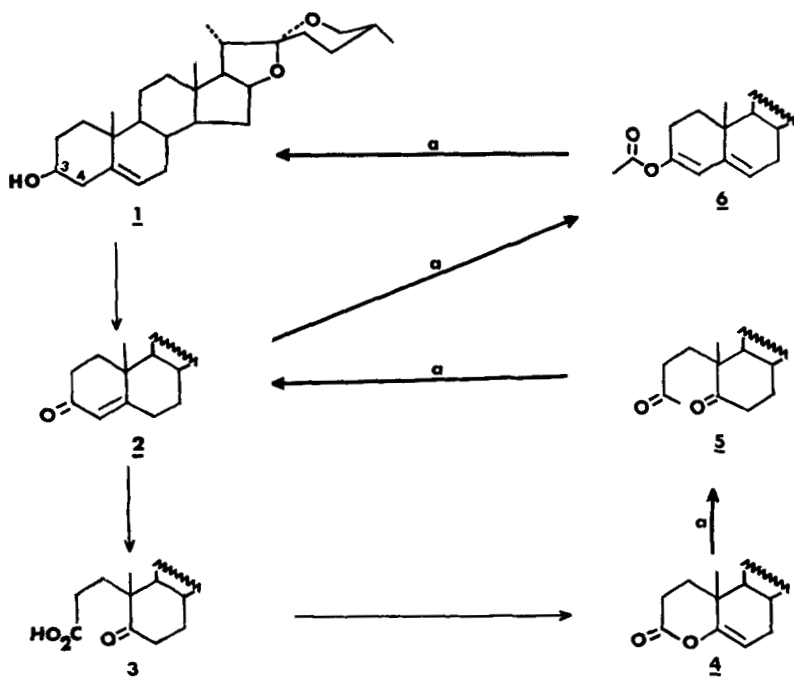
INTRODUCTION

Diosgenin, a common plant sapogenin, was found (1) to have antihypercholesterolemic properties. Metabolism studies of this compound in experimental animals had required the [¹⁴C]labelled compound, the synthesis of which is described herewith.

DISCUSSION

The reaction sequence to incorporate [¹⁴C] into the 4-position of a steroid nucleus is well known and has been described for such compounds as testosterone (2), cholesterol (3) and progesterone (4). The same approach was used to introduce [¹⁴C] into C₄ of diosgenin as shown in the reaction scheme below.

The ring A of diosgenin (**1**) was first converted to the enol lactone **4**. The sapogenin was oxidized to Δ^4 -tigogenone (**2**) (5), then, analogous to cholesterol series (2), ozonolysis of the ketone followed by lactonization with acetic anhydride and sodium acetate gave the enol lactone **4** in 32% overall yield.



^aLabelled synthesis starting with [^{14}C]methyl magnesium iodide. The label is incorporated in the 4-position of the sapogenin ring system.

The [^{14}C]label was introduced by reaction of [^{14}C]methyl magnesium iodide with the enol lactone 4 to give the corresponding diketone 5 which, on standing in acetic acid-HCl, was converted to [$4\text{-}^{14}\text{C}$] Δ^4 -tigogenone (2). Treatment of 2 with isopropenyl acetate and a trace of sulfuric acid gave the enol acetate 6 which gave [$4\text{-}^{14}\text{C}$]diosgenin (1) on reduction with sodium borohydride in methanol. The conditions were the same as those used for the conversion of Δ^4 -cholestene-3-one to cholesterol (6).

EXPERIMENTAL

[^{14}C]Methyl iodide

(20 mCi, 124 mg, specific activity 45 mCi/mmol) was purchased from New England Nuclear, Boston, Mass.

(25R)-3-Oxo-spirost-3-ene (Δ^4 -tigogenone) (2)

The unlabelled compound was prepared according to Marker et al. (5).

(25R)-5-Oxo-3,5-seco-A-norspirostan-3-oi)c)acid (3)

Δ^4 -Tigogenone (2) (40 g, 97.0 mmole) was dissolved in a mixture of ethyl acetate-acetic acid 1:1 (1.5 l) and cooled to -10° in an ice-methanol bath. Ozone (6-8% by weight, generated by a Hammond, Co. ozonator) was passed through the solution (150 ml/min) until all the ketone had reacted (ca. 4 hr). 30% Hydrogen peroxide (63 ml) was added and, after standing at 0° for 15 hr, the reaction mixture was concentrated at room temperature. Excess 10% sodium hydroxide (1.5 l) was added and the solution was stirred for 2 hr at room temperature before extracting it several times with ether to remove all neutral material. The aqueous portion was acidified with conc. HCl and repeatedly extracted with ether. The combined ether extracts were washed with water and saturated saline solution, dried over anhydrous magnesium sulphate ($MgSO_4$) and concentrated. The keto acid 3, recrystallized from ethyl acetate-methanol was obtained in 40% yield (38 g): m.p. 209-210; i.r. ($CHCl_3$) 3000, 1700 cm^{-1} ; n.m.r. ($CDCl_3$) δ 9.4 (1H, broad, exchangeable, CO_2H). Anal. Calcd. for $C_{26}H_{40}O_5$: C, 72.19; 9.32; found C, 72.18; H, 9.29.

(25R)-5-Hydroxyspirost-5-ene-3-oi)c-3,5-lactone (4)

The keto acid 3 (16 g, 37 mmole) was added to a suspension of freshly fused sodium acetate (32 g) in acetic anhydride (750 ml) and the slurry was refluxed for 18 hr. The reaction mixture was cooled, concentrated and the residue was taken up in ether. The ether extract was washed with cold 5% sodium carbonate, water and saturated saline solution, and subsequently chromatographed on a column of silica gel (2 kg), with benzene-ethyl acetate, 9:1. The enol lactone 4 (13 g) was obtained in 85% yield after recrystallization from hexane-benzene: m.p. 179-180; i.r. ($CHCl_3$) 1730, 1675 cm^{-1} ; n.m.r. ($CDCl_3$) δ 5.33 (m, 1H, =CH). Anal. Calcd. for $C_{26}H_{38}O_4$ C, 75.36; H, 9.66. Found: C, 75.43; H, 9.27.

[4-¹⁴C]- (25R)-3-Oxospirost-3-one ([4-¹⁴C]Δ⁴-tigogenon) (2)

A reaction flask containing magnesium turnings (117 mg, 4.81 mmole) and a magnetic stirrer was connected to a vacuum line, evacuated, and cooled with liquid nitrogen. [¹⁴C]Methyl iodide (124 mg, 0.874 mmole) was then condensed into the flask. The vacuum was released with dry nitrogen gas and unlabelled methyl iodide (560 mg, 3.94 mmole), dissolved in 3 ml anhydrous ether, was rapidly added from a dropping funnel. The liquid nitrogen was removed and replaced by an ice-water bath. The reaction started when the temperature rose to 0° and after 2 hr at this temperature all the magnesium had disappeared. The Grignard reagent was then transferred by means of a siphon to a dropping funnel and slowly added to a solution of the enol-lactone 4 (1.96 g, 4.83 mmole) in 50 ml anhydrous ether at 0°. The reaction mixture was stirred at room temperature for 4 hr, poured onto an ice-cold saturated ammonium chloride solution, and repeatedly extracted with ether. The combined ether extracts were washed with water and saturated saline solution, dried over MgSO₄ and concentrated. The residue was chromatographed on a column of silica gel (200 g) with benzene ethyl acetate, 9:1 to 6:4. The diketone 5 was obtained as a crystalline solid, shown to be homogeneous by TLC (0.25mm silica gel 60 F254, EM Laboratories) in benzene-ethyl acetate, 6:4.

Without further purification, the diketone 5 was dissolved in a solution of acetic acid (40ml) and concentrated HCl (3.9 ml), and left at room temperature for 14 hr under a nitrogen atmosphere. The reaction mixture was then concentrated and the residue dissolved in ethyl acetate, washed with cold 5% sodium carbonate, water and saturated saline solution, and dried over MgSO₄. After evaporation of the solvent, the title compound obtained as a crystalline solid (1.04 g, 52% yield was shown by TLC (0.25mm silica gel 60 F254; benzene-ethyl acetate, 6:4) to be homogeneous and identical with unlabelled Δ⁴-tigogenone. It was used without further purification in the next step.

[4-¹⁴C]-(25R)-3-AcetoxySpirost-3,5-diene (6).

[4-¹⁴C]Tigogenone (2) (1.04 g, 2.25 mmole), suspended in 10 ml isopropenyl acetate, was treated with two drops conc. sulfuric acid and refluxed for 20 min. After cooling to 0°, the reaction mixture was diluted with ethyl acetate and washed with 5% sodium bicarbonate, water and saturated saline solution, dried over MgSO₄ and concentrated to give the enol acetate 6 (715 mg) as a crystalline solid in 70% yield. In a pilot experiment with unlabelled tigogenone, the acetate 6 was recrystallized from hexane-ethyl acetate: m.p. 181°; i.r. (CHCl₃) 1740, 1670, 1185 cm⁻¹; n.m.r. (CDCl₃) δ 5.32 (m, 1H, =C₄H), 5.63 (m, 1H, =C₆H), 2.1 (s, 3H, COCH₃); u.v. max. (CH₃OH) 236 mμ (ε 9,000). Anal. Calcd, for C₂₆H₄₂O₄: C, 76.61; H, 9.31; found: C, 76.55; H, 9.27.

[4-¹⁴C]-(25R)-3α-Hydroxyspirost-5-ene ([¹⁴C]diosgenin) (1)

The enol acetate 6 (715 mg, 1.57 mmole) was added to a suspension of sodium borohydride (1.0 g) in 70 ml aqueous ethanol (79%) at room temperature. An additional 75 ml of aqueous ethanol and 1 g sodium borohydride was added to the reaction mixture and the solution was refluxed for 3 hr. After cooling, the mixture was diluted with water and extracted with ethyl acetate. The combined extracts were washed with water and saturated saline solution, dried over MgSO₄ and concentrated. The residue was purified by preparative TLC (0.5 mm Silica gel 60 F254 plates, EM Laboratories, using benzene-methanol, 95:5). The band corresponding to [4-¹⁴C] diosgenin was detected by autoradiography (Kodak RP/R14 Medical X-ray film, 1 hr exposure) and the material was extracted from the silica gel with hot chloroform.

The chromatographed product was obtained as a crystalline solid, m.p. 202°. Mixed melting point with pure unlabelled diosgenin (m.p. 200-202°) showed no depression. The pure [4-¹⁴C]diosgenin (395 mg), specific activity 3.77 ± 0.02 mCi/mmole, was obtained in 18% overall yield from [¹⁴C]methyl iodide.

The radiochemical purity of the compound was determined by TLC-autoradiography in three different solvent systems: benzene-methanol, 95:5; hexane-ethyl

acetate, 2:l; hexane-ether-acetic acid, 90:l. After locating the radioactive zone(s) on the TLC plate from the autoradiogram, the silica gel was scraped from each plate in 1 cm strips and counted as described by Shaw et al. (7). The radiochemical purity of the [4-¹⁴C] diosgenin was > 99.7% in all three solvent systems.

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