Synthesis of [4-^{14}C]Ergosterol and [4-^{14}C]Ergocalciferol

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[4-^{14}C]Ergosterol has been prepared from ergosta-4,22-dien-3-one *via* 4-oxaergosta-5,22-dien-3-one; the intermediate [4-^{14}C]ergosta-4,22-dien-3-one has been converted into [4-^{14}C]ergocalciferol.

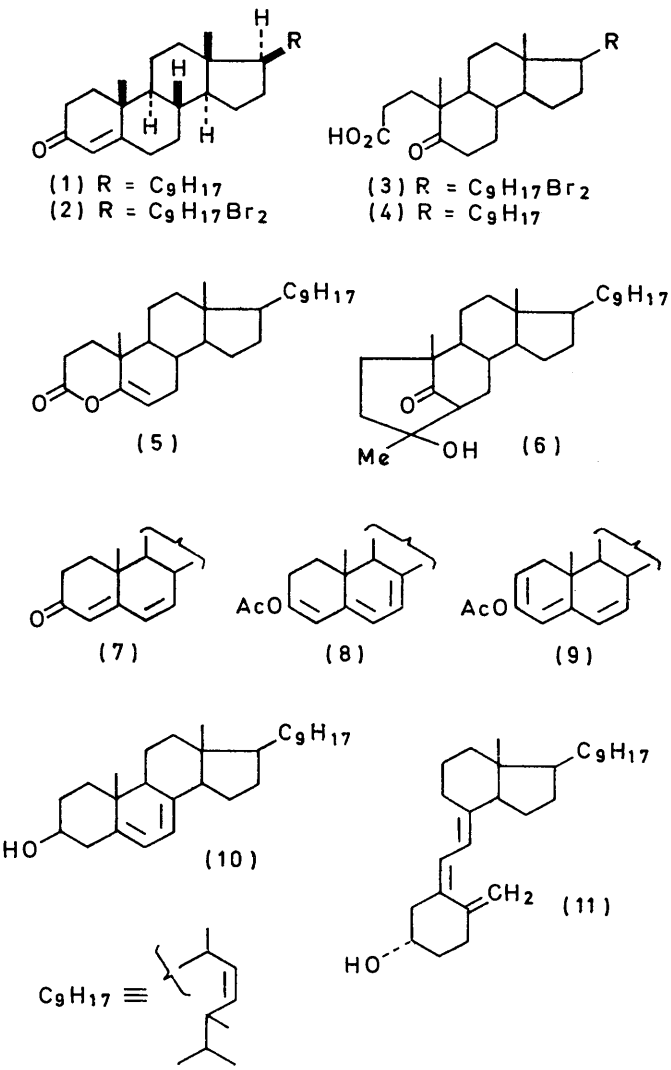
PREPARATIONS of generally labelled ergocalciferol (Wilzbach tritium-labelling method and a biochemical synthesis from [^{14}C]acetate) have been described previously.[1-3] Recently we have reported syntheses of [1-^{3}H]ergosterol and [1-^{3}H]ergocalciferol [4] and a synthesis of [*G*-^{3}H]ergosterol by catalytic tritium exchange in aqueous dimethylformamide.[5] The advantage of ^{3}H,^{14}C-double labelling in biochemical studies [6] has now led us to prepare [4-^{14}C]ergocalciferol for studies of the intermediary metabolism of vitamin D_2.

Ergosta-4,22-dien-3-one (1) was selectively brominated to give the 22,23-dibromo-derivative (2). The u.v. absorption ($\lambda_{max.}$ 242 nm) showed the presence of the unchanged 4-en-3-one group. Ozonolysis [7] of compound (2) afforded the dibromo-acid (3), which was debrominated with zinc dust in ether–acetic acid. Only partial debromination was observed at ambient temperature, but brief treatment under reflux gave the halogen-free acid (4), $\nu_{max.}$ 1700 (CO) and 2600—3200 cm^{-1} (CO_2H). Treatment of this acid with acetic anhydride and toluene-*p*-sulphonic acid [8] gave the 4-oxa-3-oxo-derivative (5), $\nu_{max.}$ 1755 and 1155 cm^{-1} (lactone), in moderate yield.

Reaction of the lactone (5) with methylmagnesium iodide [9-11] led to the keto-alcohol (6), $\nu_{max.}$ 1710 cm^{-1} (CO) and 3450 cm^{-1} (OH), which on treatment with alkali gave back the ergostadienone (1).

In a similar way the [4-^{14}C]ergostadienone was prepared from the lactone (5) and [^{14}C]methyl iodide; the product had a specific activity of 52 mCi mmol^{-1}. By use of the procedure worked out for the preparation of [1-^{3}H]ergosterol from [1,2-$^{3}H_2$]ergosta-4,22-dien-3-one,[4] the [4-^{14}C]ergostadienone was converted into [4-^{14}C]-isoergosterone (7) on reaction with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in dioxan containing hydrogen chloride. Labelled isoergosterone gave a mixture of enol acetates (8) and (9) on reaction with acetic anhydride and sulphosalicylic acid in toluene and this mixture was reduced with sodium borohydride to give [4-^{14}C]ergosterol (10).

Irradiation of an ethereal solution of labelled ergosterol in a quartz vessel with a 100 W medium-pressure mercury arc under nitrogen [4] gave a mixture of products from which pre-ergocalciferol and tachysterol$_2$ were isolated by preparative t.l.c. on silica gel. The pre vitamin was converted into [4-^{14}C]ergocalciferol (11) by



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thermal equilibration in benzene and finally purified by preparative t.l.c.

EXPERIMENTAL

M.p.s were determined with a Gallenkamp apparatus. I.r. and u.v. spectra were recorded with Unicam SP 200 and 800 spectrometers. Kieselgel GF_{254} (Merck) was used for t.l.c. plates. Radioactivity was measured by scintillation counting of samples (5—10 mg each) in a Packard Tri-Carb model 3375 liquid-scintillation instrument.

22,23-*Dibromoergost*-4-*en*-3-*one* (2).—Ergosta-4,22-dien-3-one (1) (2 g) in ether (30 ml) and pyridine (1 ml) was treated with a solution of bromine (810 mg) in acetic acid (8 ml) for 5 min. Water was added; the product was extracted into ether and the extract was washed with dilute sodium hydroxide solution and water. Evaporation gave the *dibromo-ketone* (2) (1·3 g), m.p. 205—207° (from acetone–methanol), $\lambda_{max.}$ (EtOH) 242 nm (ε 14,300) (Found: C, 60·3; H, 8·0; Br, 28·5. $C_{28}H_{44}Br_2O$ requires C, 60·4; H, 8·0; Br, 28·7%).

4-*Oxaergosta*-5,22-*dien*-3-*one* (5).—The dibromo-ketone (2) (1·2 g) in ethyl acetate (30 ml) and acetic acid (10 ml) in an ice–salt bath was treated with a stream of ozone until a sample showed no absorption at 242 nm (*ca.* 1 h). Water (5 ml) and hydrogen peroxide (30%; 1 ml) were added and the mixture was left overnight. The product was extracted with dilute sodium hydroxide solution; after acidification, the dibromo-acid (3) was extracted into ether (20 ml). The solution was stirred with zinc dust (1 g) and acetic acid (2 ml) for 2 h. The zinc dust was filtered off and washed with ether, and the solution was concentrated to the original volume. Fresh zinc dust (1 g) was added and the mixture was refluxed for 30 min; the usual work-up then gave the halogen-free acid (4).

The acid (4) in acetic anhydride (10 ml) and toluene-*p*-sulphonic acid (80 mg) were heated at 90° for 3 h. The product was extracted into ether; the extract was washed with sodium hydrogen carbonate solution and water, dried, and filtered through a silica gel column (20 g). Final t.l.c. purification gave the *unsaturated lactone* (5), m.p. 129—131° (from acetone) (Found: C, 81·5; H, 10·9. $C_{27}H_{42}O_2$ requires C, 81·4; H, 10·6%).

Ergosta-4,22-*dien*-3-*one* (1).—The lactone (5) (100 mg) in ether (5 ml) was added to methylmagnesium iodide (1 mol. equiv.) in ether (5 ml) under nitrogen, and the solution was left overnight. Ether and dilute hydrochloric acid were added and the usual work-up gave the semicrystalline derivative (6). This was dissolved in methanol (25 ml), potassium hydroxide (800 mg) was added, and the solution was heated under reflux for 2 h. Extraction with ether and t.l.c. purification afforded the ketone (1) (53 mg); u.v. analysis showed 95% purity.

[4-^{14}C]*Ergosta*-4,22-*dien*-3-*one* (1).—Use of [^{14}C]methyl iodide (*ca.* 30 mCi) in the foregoing reaction gave labelled ketone (1) (60 mg), specific activity 52 mCi mmol^{-1}.

[4-^{14}C]*Ergosterol* (10).—By use of the procedure described for the preparation of [1-^{3}H]ergosterol,[4] the [4-^{14}C]ketone (1) was converted into [4-^{14}C]isoergosterone (7) and thence into a mixture of enol acetates (8) and (9). Reduction with sodium borohydride afforded [4-^{14}C]ergosterol (10 mg), specific activity 49 mCi mmol^{-1}.

[4-^{14}C]*Ergocalciferol* (11).—A solution of [4-^{14}C]ergosterol (10) (10 mg) in ether (100 ml), in a quartz apparatus cooled in ice, was agitated with a stream of oxygen-free nitrogen, dispersed through a sintered glass disc in the base of the vessel. The solution was irradiated for 12 min, then evaporated to dryness under nitrogen below 15°. The residue dissolved in chloroform was applied to a silica gel plate (200 × 200 × 1 mm) and the plate was developed twice in chloroform. The previtamin and tachysterol bands were located under u.v. light, scraped off, and eluted with ether. The starting material was irradiated once more for 12 min and the previtamin and tachysterol fractions were combined with the first crops. The solvent was evaporated off and the previtamin, dissolved in benzene and methanol (9 : 1; 10 ml), was refluxed under nitrogen for 2 h. The solvent was evaporated off under nitrogen and the residue applied to a silica gel plate which was developed twice in chloroform. The vitamin fraction was eluted with ether. The previtamin fraction was once more equilibrated. [4-^{14}C]Ergocalciferol (11) (1·62 mg; specific activity 48 mCi mmol^{-1}) was obtained.

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