[*G*-^{3}H]Ergosterol; Location of Tritium in Rings A and B

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[*G*-^{3}H]Ergosterol was prepared from ergosterone by catalytic tritium exchange in aqueous dimethylformamide on a platinum catalyst, followed by enol acetylation and reduction with sodium borohydride. The tritium was found to be distributed 41% at the 1α-, 1β-, 2- and 4-positions, 29% at the 6- and 7-positions, and 30% in the remaining part of the molecule.

As a counterpart for biochemical studies with the recently described [1-^{3}H]ergocalciferol,[1] a generally tritium-labelled ([*G*-^{3}H]) ergocalciferol has been considered. The tritium labelling of 4-en-3-ones by column chromatography of ergosterone (1) on alkaline alumina containing tritiated water [2] offered a possibility of making a 2- and 4-tritiated compound, but could not be used in the preparation of tritiated ergosterol, where high radiochemical activity would be desired. Wilzbach's method of tritiation [3] did not offer any high radiochemical activity either and so our attention was turned towards tritium labelling by catalytic exchange in aqueous solvents.[4]

Ergosterone (1) was heated with dimethylformamide,

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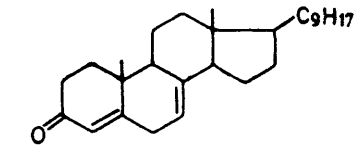
[1] B. Pelc and E. Kodicek, *J. Chem. Soc.* (*C*), 1971, 3415.

[2] M. J. Thompson, O. W. Berngruber, and P. D. Klein, *Lipids*, 1971, **6**, 233.

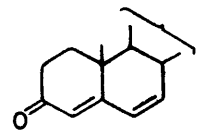
[3] K. E. Wilzbach, *J. Amer. Chem. Soc.*, 1957, **79**, 1013.

[4] Tritium labelling service, Amersham, Technical bulletin 71/3.

tritiated water, and prereduced Adams catalyst at *ca.* 120° for 20 h. Preparative t.l.c. purification separated [*G*-^{3}H]ergosterone (1) and [*G*-^{3}H]isoergosterone (2) in the ratio 61 : 13, with 0·65% of tritium incorporation



(1)



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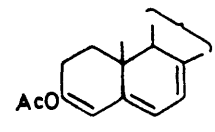

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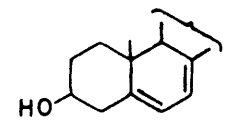

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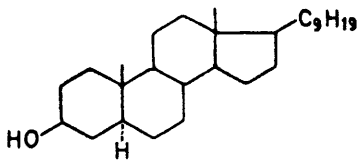

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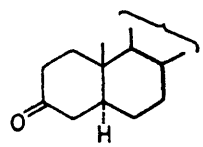

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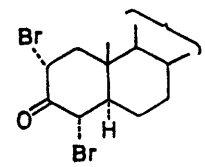

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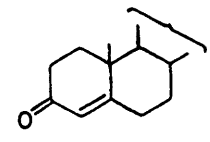

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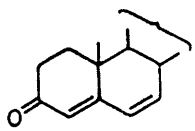

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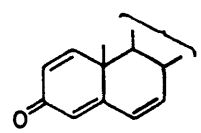

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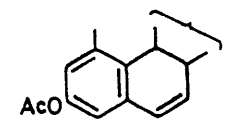

(11)

in the former compound. Ergosterone was converted into the enol acetate (3) with acetic anhydride and sulphosalicylic acid, and reduced to [*G*-^{3}H]ergosterol (4) with sodium borohydride [1] without any considerable loss of activity (40 μCi mmol^{-1}).

For the tritium distribution experiments, [*G*-^{3}H]-ergosterol was diluted with the carrier, recrystallised, and hydrogenated to give 5α-ergost-8(14)-en-3β-ol.[5,6] Isomerisation to 5α-ergost-14-en-3β-ol and hydrogenation afforded the saturated derivative (5).[2,7-9] No loss of radioactivity was observed in any of these steps (Table). Oxidation with sodium dichromate dihydrate in acetic acid–sulphuric acid [10] gave the ketone (6), with a loss of 31% of the specific molar activity. Since there is no exchangeable hydrogen at C-3 in structure (1), tritium must have been lost from positions 2 and 4. This was confirmed when attempted drastic alkaline equilibration [11] did not result in any other loss of radioactivity.

Bromination of the ketone (6) gave the 2α,4α-dibromo-derivative (7); [5] this was converted into ergost-4-en-3-one (8) by treatment with sodium iodide followed by reductive dehalogenation with zinc.[12,13] A slight loss of radioactivity (8%; probably from position 6) was observed in this step, probably caused by the presence of acetic acid during dehalogenation of the intermediate 2-iodo-derivative. Drastic alkaline equilibration [11] caused further loss of radioactivity (13%) from position 6. Dehydrogenation of the ketone (8)

Relative activity of derivatives of [*G*-^{3}H] ergosterol

	10^3 disint. min^{-1}	%	Specific loss
Ergosterol (4)	375	102	
5α-Ergost-8(14)en-3β-ol	369	100	
5α-Ergost-14-en-3β-ol (5)	368	100	
5α-Ergostan-3-one (6)	253	69	31% from positions 2 and 4
5α-Ergostan-3-one (6) *	250	68	
2α,4α-Dibromoergostan-3-one (7)	252	69	
Ergost-4-en-3-one (8)	224	61	8% from position 6
Ergost-4-en-3-one (8) *	178	48	13% from position 6
Ergosta-4,6-dien-3-one (9)	149	40	8% from position 7α
Ergosta-1,4,6-trien-3-one (10)	120	33	7% from position 1α
3-Acetoxy-1-methylergosta-1,3,5(10)6-tetraene (11)	110	30	3% from position 1β

Standard deviations were ±1%.

* After alkaline equilibration.

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[8] J. W. Cornforth, I. Y. Gore, and G. Popjak, *Biochem. J.*, 1957, **65**, 94.
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[11] Y. Osawa and D. G. Spaeth, *Biochemistry*, 1971, **10**, 66.
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with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) [14] gave the dienone (9) with a stereospecific loss of tritium from position 7α. Subsequent dehydrogenation of compound (9) to the trienone (10) caused stereospecific loss of tritium from position 1α, and conversion of the trienone (10) into the aromatic compound (11) indicated that 3% of tritium had been at position 1β. The remaining 30% of radioactivity must have been at position 7β and other positions in the molecule.

The tritium-distribution experiments indicated that at least 70% of the tritium is located in rings A and B. Dehydrogenation of ergosterone with DDQ[15] showed that enolisation of the 3-oxo-group in ergosterone (1) occurs towards C-6 rather than C-2. We conclude that the majority of tritium in ring A is associated with C-4 rather than with C-2, and the majority of the total tritium is located at C-4 and C-6 of ergosterone.

EXPERIMENTAL

U.v. spectra were recorded with a Unicam SP 200 spectrometer. Radioactivity was measured by scintillation counting of samples (5—10 mg) in a Packard Tri-Carb model 3375 liquid-scintillation spectrometer.

Tritiation of Ergosterone.—Ergosterone (1) (100 mg) in 90% dimethylformamide (3 ml), tritiated water (0·2 ml; 1 mCi), and prereduced Adams catalyst (20 mg) were heated under reflux for 20 h. Water was added and the product was extracted into ether and washed with water. Preparative t.l.c. (silica gel) gave [*G*-^{3}H]ergosterone (1) (61 mg), specific molar activity 42 μCi mmol^{-1}, 97% purity (u.v. analysis). A more polar fraction (13 mg) was identified as [*G*-^{3}H]isoergosterone (2) (u.v. analysis at 285 nm).

[*G*-^{3}H]*Ergosterol* (4).—[*G*-^{3}H]Ergosterone (1) (61 mg) in toluene (20 ml), acetic anhydride (0·5 ml), and sulphosalicylic acid (10 mg) were heated under reflux for 3 h; toluene was then distilled off slowly. Pyridine (1 drop) was added to the residue, which was then evaporated under reduced pressure with periodic additions of ethanol and toluene. The residue in benzene was filtered through a silica gel column (3 g); evaporation gave the enol acetate (3) (52 mg), $\lambda_{max.}$ 301, 304, and 328 nm. A solution of this in ether (1 ml) and 90% ethanol (3 ml) was reduced with sodium borohydride (100 mg) overnight at 0—5°. After hydrolysis [5N-HCl (1 ml); reflux for 30 min] the product was extracted into ether and purified by t.l.c. to give ergosterol (4) (32 mg), specific molar activity 40 μCi mmol^{-1} (u.v. analysis showed 95% purity).

5α-*Ergostan*-3β-*ol* (5).—[*G*-^{3}H]Ergosterol was diluted with ergosterol, recrystallised from ethanol, and hydrogenated according to the method of Nes and Mosettig.[6] The product was isomerised according to the method of Cornforth *et al.*[8] and hydrogenated to give ergostanol (5), m.p. 135—140° (lit.,[7] 143—144°).

5α-*Ergostan*-3-*one* (6).—Ergostanol (5) (3 g) in benzene (50 ml) was stirred with sodium dichromate dihydrate (5·1 g) in acetic acid (3·8 ml), sulphuric acid (8·3 ml), and water (23 ml) for 6 h at 20—25°. After the usual work-up, a solution of the product in light petroleum (b.p. 40—60°) was filtered through alumina (30 g; activity II) and eluted with light petroleum (*ca.* 900 ml). Two crystallisations afforded compound (6) (1·5 g), m.p. 158—160° (from ethyl acetate–methanol) (lit.,[9] m.p. 160°).

2α,4α-*Dibromoergostan*-3-*one* (7).—The ketone (6) was brominated;[5] crystallisation from acetone gave the dibromo-derivative (7), m.p. 196—198° (lit.,[5] 194—195°).

Ergost-4-*en*-3-*one* (8).—The dibromo-derivative (7) (510 mg) in acetone (20 ml) and sodium iodide (1 g) were heated under reflux for 20 h. Water was added and the product was extracted into ether. The extract was washed with sodium thiosulphate solution and water. The solution was stirred with zinc dust (2 g) and a few drops of acetic acid for 5 h. The material was purified by preparative t.l.c. and crystallised from 90% ethanol to give *compound* (8) (160 mg), m.p. 95—97°, $\lambda_{max.}$ (EtOH) 242 nm (ε 13,800) (Found: C, 84·6; H, 11·3. $C_{28}H_{46}O$ requires C, 84·4; H, 11·6%).

Alkaline equilibration [potassium hydroxide (200 mg), 90% ethanol (10 ml), and benzene (10 ml)] under reflux for 3 h resulted in loss of 13% of tritium.

Ergosta-4,6-*dien*-3-*one* (9).—The ketone (8) (100 mg), DDQ (65 mg), and dioxan (10 ml) were treated for a few seconds with dry hydrogen chloride and the mixture was left for 2 h at 25°. Sodium thiosulphate solution was added and the product was extracted into ether. The extract was washed with sodium hydrogen carbonate solution and water. T.l.c. and crystallisation from 90% ethanol gave *compound* (9), m.p. 79—81°, $\lambda_{max.}$ (EtOH) 285 nm (ε 21,000) (Found: C, 84·9; H, 10·9. $C_{28}H_{44}O$ requires C, 84·8; H, 11·2%).

Ergosta-1,4,6-*trien*-3-*one* (10).—The ketone (10) (60 mg), DDQ (35 mg), toluene-*p*-sulphonic acid (100 mg), and dioxan (5 ml) were heated under reflux for 3 h. After the usual work-up the product was purified by t.l.c. and characterised [$\lambda_{max.}$ 225, 257, and 299 nm (ε 12,000, 10,800 and 12,300) [5]].

The ketone (10) (30 mg) was converted into the aromatic compound (11),[5] which was purified on alumina to give amorphous material (15 mg), $\lambda_{max.}$ 224 and 265 nm (ε 22,500 and 6800).

We thank the University Chemical Laboratory, Cambridge, for microanalyses.

[2/681 *Received*, 21*st March*, 1972]

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