The Location of Tritium in [1-^{3}H]Ergosterol

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Tritium in [1-^{3}H]ergosterol has been found to be distributed 83—85% at the 1α- and 13—15% at the 1β-position. It has been confirmed that the tritium at the 2-position is lost during enol acetylation of [1.2-$^3H_2$]ergosta-4,6.22-trien-3-one. A comparison with other 1- and 1,2-tritiated steroids is given.

THE determination of tritium distribution in steroids labelled for biochemical studies has been well documented.[1–14] Compounds labelled with tritium in the 1-position have been prepared from Δ^1- or $\Delta^{1,4}$-3-oxo-derivatives by heterogeneous [15] or homogeneous [16,17] [1-^{3}H]ergosterol [18] in view of the difficulties of applying stereochemical degradative reactions to ergocalciferol itself.

[1-^{3}H]Ergosterol was prepared from ergosta-1,4,22-trien-3-one by hydrogenation with tritium over the homogeneous catalyst, chlorotris(triphenylphosphine)-rhodium(I). The resulting [1,2-$^3H_2$]ergosta-4,22-dien-3-one (specific activity 2·4 Ci mmol^{-1}) was converted into the 4,6,22-trien-3-one (2·4 Ci mmol^{-1}) and this on

TABLE 1

Relative distribution of tritium in [1-^{3}H]steroids

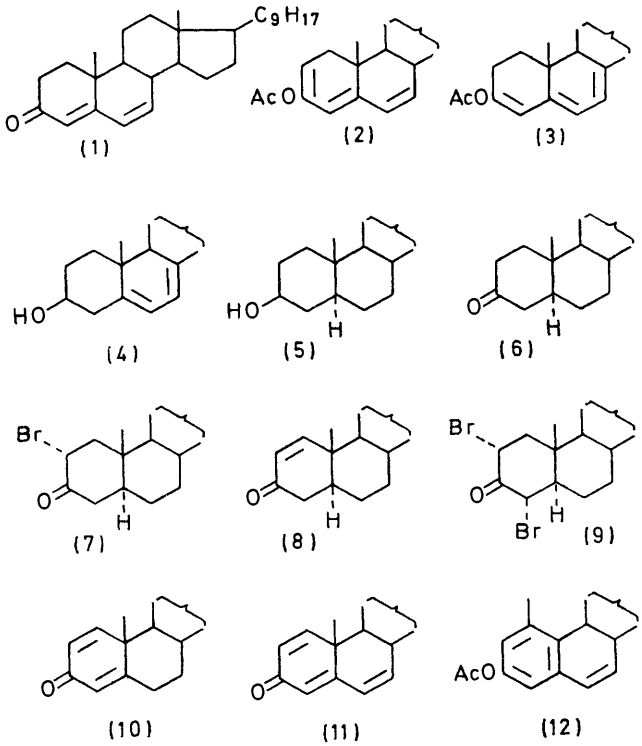
No.	Derivative	Position of label (%) 1α-	1β-	2α-	2β-	Other	Starting material–catalyst
1	[1-^{3}H]Cholesterol [8,13]	83—85	13—15			2	1-en-3-one–Pd [a]
2	[1-^{3}H]Ergosterol [18]	83—85	13—15			2	1,4-dien-3-one–Rh [b]
3	[1-^{3}H]Androst-4-ene-3,17-dione [1]	25	75				1,4-diene-3,17-dione–Pd [a]
4	[1-^{3}H]Androst-4-ene-3,17-dione [1]	93	7				1-ene-3,17-dione–Pd [a]
5	[1-^{3}H]Testosterone [2]	17	83				1,4-dien-3-one–Pd [a]
6	[1,2-$^3H_2$]Cholesterol [9,13]	47—49	15—17	α + β 36			1,4-dien-3-one–Rh [b]
7	[1,2-$^3H_2$]Cholesterol [6,7,13]	38—44	16—22	α + β 40			1,4-dien-3-one–Pd [a]
8	[1,2-$^3H_2$]Testosterone [4]	13	44	18	25		1,4-dien-3-one–Pd [b]
9	[1,2-$^3H_2$]Testosterone [10]	43	7	43	6	1	1,4-dien-3-one–Rh [b]
10	[1,2-$^3H_2$]Testosterone [10]	13	38	12	37		1,4-dien-3-one–Pd [a]
11	[1,2-$^3H_2$]-11β-Hydroxyandrost-4-ene-3,17-dione [4]	16	53·5	α + β 21		9·5	From [1,2-$^3H_2$]hydrocortisone (Pd) after cleavage
12	[1,2-$^3H_2$]-11β-Hydroxyandrost-4-ene-3,17-dione [4]	13	38	α + β 42		7	1,4-diene-3,17-dione–Pd [a]

[a] Pd–C. [b] Chlorotris(triphenylphosphine)rhodium(I).

hydrogenation (Table 1). The nature of the hydrogenation determines the ratio of tritium in the α- and β-configurations. We have studied the distribution in

[1] H. J. Brodie, M. Hayano, and M. Gut, *J. Amer. Chem. Soc.*, 1962, **84**, 3766.
[2] H. J. Brodie, *Tetrahedron*, 1967, **23**, 535.
[3] S. Baba, H. J. Brodie, M. Hayano, G. Kwass, and M. Gut, *J. Org. Chem.*, 1964, **29**, 2751.
[4] H. J. Brodie, K. Raab, G. Possanza, N. Seto, and M. Gut, *J. Org. Chem.*, 1969, **34**, 2697.
[5] H. J. Brodie, S. Baba, M. Gut, and M. Hayano, *Steroids*, 1965, **6**, 659.
[6] M. R. Haussler, J. F. Myrtle, and A. W. Norman, *J. Biol. Chem.*, 1968, **243**, 4055.
[7] P. F. Neville and H. F. DeLuca, *Biochemistry*, 1966, **5**, 2201.
[8] R. K. Callow, E. Kodicek, and G. A. Thompson, *Proc. Roy. Soc.*, 1966, *B*, **164**, 1.
[9] D. E. M. Lawson, B. Pelc, P. A. Bell, P. W. Wilson, and E. Kodicek, *Biochem. J.*, 1971, **121**, 673.
[10] Y. Osawa and D. G. Spaeth, *Biochemistry*, 1971, **10**, 66.
[11] J. Fishman and H. Guzik, *J. Amer. Chem. Soc.*, 1969, **91**, 2805.
[12] J. Fishman, H. Guzik, and D. Dixon, *Biochemistry*, 1969, **8**, 4304.
[13] P. A. Bell and E. Kodicek, *Biochem. J.*, 1970, **116**, 755.
[14] S. Burstein, H. L. Kimball, and M. Gut, *Steroids*, 1966, **8**, 789.
[15] D. Osinski and H. Vanderhaeghe, *Rec. Trav. chim.*, 1960, **79**, 216.
[16] C. Djerassi and J. Gutzwiller, *J. Amer. Chem. Soc.*, 1966, **88**, 4573.
[17] A. J. Birch and K. A. M. Walker, *J. Chem. Soc.* (*C*), 1966, 1894.

enol acetylation gave a mixture of the 3-acetoxy-2,4,6,22-tetraene and the 3-acetoxy-3,5,7,22-tetraene (1·3 Ci mmol^{-1}). Reduction of this mixture with sodium borohydride gave [1-^{3}H]ergosterol (1·3 Ci mmol^{-1}) and [1-^{3}H]-ergosta-4,6-dien-3β-ol, purified by preparative t.l.c.[18]



It was not clear whether the mixture of enol acetates contained the [1 ^{3}H]- or the [1,2-$^3H_2$]-3-acetoxy-3,5,7,22-tetraene, but the tritium-distribution experiments showed that ergosterol contained the label in the 1-position exclusively. To confirm the loss of tritium from the 2-position during enol acetylation, isoergosterone (1) was converted into the 3-acetoxy-2,4,6,22-tetraene (2) with isopropenyl acetate and toluene-*p*-sulphonic acid in benzene.[19] This tetraene gave, after heating under reflux in toluene–acetic anhydride with sulphosalicylic acid [5] (as for the radioactive material), the isomeric 3-acetoxy-3,5,7,22-tetraene (3). Attempted isomerisation in toluene under reflux or in toluene–acetic anhydride under reflux was not successful. This showed that the isomerisation is not a thermally equilibrated reaction, that the 3-acetoxy-2,4,6,22-tetraene is the primary product of enol acetylation, and that tritium is lost from the 2-position.

For the tritium-distribution experiments, [1-^{3}H]-ergosterol was diluted with the carrier, trienol (4), recrystallised, and hydrogenated to give 5α-ergost-8(14)-en-3β-ol.[20,21] Isomerisation to 5α-ergost-14-en-3β-ol and hydrogenation in ether–acetic acid gave the saturated alcohol (5).[22-24] Oxidation with sodium dichromate dihydrate afforded the ketone (6); [24] attempted equilibration of which with potassium hydroxide in aqueous methanol for 20 h or under reflux for 3 h [10] did not cause any loss of radioactivity, thus confirming that there was no tritium at the 2-position.

Monobromination of ketone (6) [25] gave 2α-bromo-compound (7), which was dehydrobrominated with calcium carbonate in dimethylformamide [26] to the unsaturated compound (8) with a stereospecific loss of tritium from the 1α-position. The dehydrogenation with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) [27] of the 3-oxo-compound (6) gave the same result. Dibromination of the ketone (6) [25] afforded the 2α,4α-dibromo-derivative (9). Subsequent stereospecific dehydrobromination with calcium carbonate in dimethylformamide gave the dienone (10). This was purified on a column and by t.l.c. and converted into the 1,4,6-trien-3-one (11),[27] which showed a loss of radioactivity similar to that for the enone (8). A dienone–phenol rearrangement [28] gave the amorphous aromatic product (12) with retention of *ca.* 2% of the original radioactivity. The results are summarised in Table 2.

TABLE 2 [a]

Relative molar activity (r.m.a.) of derivatives of [1-^{3}H]ergosterol

Derivative	R.m.a. (%)
Ergosterol	102
5α-Ergost-8(14)-en-3β-ol	102
5α-Ergostan-3β-ol	101
5α-Ergostan-3-one	100
5α-Ergostan-3-one [b]	100
2α-Bromo-5α-ergostan-3-one	100
5α-Ergost-1-en-3-one [c]	17
5α-Ergost-1-en-3-one [d]	16
2α,4α-Dibromo-5α-ergostan-3-one	99
Ergosta-1,4,6-trien-3-one [e]	15
3-Acetoxy-1-methyl-19-norergosta-1,3,5(10),6-tetraene	2

[a] Standard deviations were less than 1%. [b] After alkaline equilibration. [c] After dehydrobromination of the 2α-bromo-derivative. [d] After dehydrogenation with DDQ of the 3-oxo-derivative. [e] Dehydrobromination of the 2α,4α-dibromo-derivative, followed by dehydrogenation with DDQ.

We found that the relative distribution of tritium in [1-^{3}H]ergosterol (no. 2, Table 1) is very close to that of [1-^{3}H]cholesterol (no. 1). Comparison with the known [1-^{3}H]- and [1,2-$^3H_2$]-steroids shows some differences. When Δ^1-3-oxo-steroids were used as starting materials (nos. 1 and 4) the relative distribution of tritium in the

[18] B. Pelc and E. Kodicek, *J. Chem. Soc.* (*C*), 1971, 3415.
[19] R. B. Moffett and D. I. Weisblat, *J. Amer. Chem. Soc.*, 1952, **74**, 2183.
[20] A. J. Liston, P. Toft, P. Morand, and H. Stollar, *J. Chem. Soc.* (*C*), 1970, 2121.
[21] W. R. Nes and E. Mosettig, *J. Org. Chem.*, 1953, **18**, 276.
[22] I. M. Heilbron and D. G. Wilkinson, *J. Chem. Soc.*, 1932, 1708.
[23] J. W. Cornforth, I. Y. Gore, and G. Popjak, *Biochem. J.*, 1957, **65**, 94.
[24] D. H. R. Barton, J. D. Cox, and N. J. Holness, *J. Chem. Soc.*, 1949, 1771.
[25] C. Djerassi and C. R. Scholz, *J. Amer. Chem. Soc.*, 1946, **68**, 1712; 1947, **69**, 2404.
[26] J. A. Zderic, H. Carpio, A. Bowers, and C. Djerassi, *Steroids*, 1963, **1**, 233.
[27] A. B. Turner and H. J. Ringold, *J. Chem. Soc.* (*C*), 1967, 1720.
[28] J. Romo, C. Djerassi, and G. Rosenkranz, *J. Org. Chem.*, 1950, **15**, 869.

1α- and 1β-positions was more or less the same. This difference could be explained in terms of a long-range effect of the 17-substituents, though there are not yet many examples available. Hydrogenation of 1,4-dien-3-ones on a heterogeneous catalyst gave predominantly β-tritiated steroids (with the exception of cholesterol, no. 7) and did not cause equal labelling at the 1- and 2-positions (nos. 8, 11, and 12 show that there was more tritium at the 1- than the 2-position). But Osawa and Spaeth [10] found that hydrogenation did label both positions equally (*e.g.*, nos. 9 and 10), not only with a homogeneous catalyst but also with a heterogeneous catalyst. The extreme lability of a 2-tritium atom is the cause of the easy loss of part of label during purification of [1,2-$^3H_2$]-steroids.[10]

EXPERIMENTAL

M.p.s were taken on a Gallenkamp apparatus. U.v. spectra were recorded with a Unicam SP 200 spectrometer, and optical rotations were measured for solutions in chloroform (*c* 0·5%) at 20—25°. Radioactivity was measured by scintillation counting of multiple samples (5—10 mg each) in a Packard Tri-Carb model 3375 liquid-scintillation spectrometer.

3-*Acetoxyergosta*-2,4,6,22-*tetraene* (2).—Isoergosterone (1) (500 mg), benzene (50 ml), and isopropenyl acetate (15 ml) were heated under reflux for 24 h with toluene-*p*-sulphonic acid (50 mg); the distillate was dried with silica gel. The solvents were evaporated *in vacuo* and the residue was dissolved in light petroleum–benzene (1 : 1) and filtered through silica gel (10 g). The product crystallised from ethanol to give the tetraene (2) (330 mg), m.p. 137—139°, $\lambda_{max.}$ (MeOH) 302 nm (ε 15,000), [lit.,[29] m.p. 137°, $\lambda_{max.}$ 304 nm (ε 16,500)].

3-*Acetoxyergosta*-3,5,7,22-*tetraene* (3).—The enol acetate (2) (50 mg), sulphosalicylic acid (5 mg), toluene (5 ml), and acetic anhydride (0·5 ml) were heated under reflux for 3 h. The solvents were evaporated off *in vacuo* and, after the usual work-up, the product was crystallised from ethanol; m.p. 143—145° (lit.,[29] 146°). U.v. analysis (at 314 nm) showed 85% of enol acetate (3).

5α-*Ergostan*-3-*one* (6).—Ergosterol (4) (10 g) was hydrogenated according to the method of Nes and Mosettig,[21] to give 5α-ergostan-3β-ol(5),[22] which was oxidised to the ketone (6) (3·1 g), m.p. 156—159° (from methanol–ethyl acetate), (lit.,[24] 160°).

2α-*Bromo*-5α-*ergostan*-3-*one* (7).—The ketone (6) (1 g) in acetic acid (35 ml), containing few drops of a solution of hydrogen bromide in acetic acid, was brominated with bromine (400 mg) in acetic acid (3·6 ml). Methanol (20 ml) was added and the product was left in the refrigerator overnight. The product was washed with methanol and ether to give the bromo-derivative (7) (700 mg), m.p. 179—181° (from acetone) (lit.,[30] 191°), $[\alpha]_D$ +30° (Found: C, 70·4; H, 9·8; Br, 16·1. Calc. for $C_{28}H_{47}BrO$: C, 70·1; H, 9·9; Br, 16·6%).

5α-*Ergost*-1-*en*-3-*one* (8).—(*a*) The bromo-derivative (7) (600 mg) and calcium carbonate (1 g) in dimethylformamide (10 ml) were heated under reflux for 1 h. The product was extracted into benzene, washed with water, and chromatographed on alumina (15 g; activity II). Elution with light petroleum–benzene (2 : 1) gave the *ketone* (8) (250 mg), m.p. 119—121° (from acetone–methanol) $[\alpha]_D$ +43°, $\lambda_{max.}$ (MeOH) 230 nm (ε 9400) (Found: C, 84·2; H, 11·8. $C_{28}H_{46}O$ requires C, 84·4; H, 11·6%).

(*b*) The ketone (6) (200 mg) and DDQ (135 mg) in dioxan (10 ml) were heated under reflux for 24 h. Ether extracts were washed with sodium thiosulphate solution, sodium hydrogen carbonate solution, and water. The product was purified by t.l.c. (silica gel) to 94% purity (u.v. analysis at 230 nm).

2α,4α-*Dibromoergostan*-3-*one* (9).—The ketone (6) (1 g) in acetic acid (35 ml) and carbon tetrachloride (15 ml) containing few drops of a solution of hydrogen bromide in acetic acid, was treated with a solution of bromine (800 mg) in acetic acid (7·2 ml) for 24 h. Water was added, and the product was extracted into benzene and washed with dilute sodium hydroxide solution and water to give the *dibromo-derivative* (9) (1·1 g), m.p. 194—195° (from acetone), $[\alpha]_D$ −7°, (Found: C, 59·9; H, 8·3; Br, 28·0. $C_{28}H_{46}Br_2O$ requires C, 60·2; H, 8·3; Br, 28·6%).

Ergosta-1,4-*dien*-3-*one* (10).—The dibromo-derivative (9) (1 g) and calcium carbonate (1 g) in dimethylformamide were heated under reflux for 1 h. The product was extracted into benzene and washed with water. After evaporation, the residue was chromatographed on alumina (30 g; activity II). Elution with light petroleum–benzene (1 : 1) and benzene gave material which resisted all attempts at crystallisation. It was pure (by t.l.c. and g.l.c.) and had $\lambda_{max.}$ (EtOH) 245 nm (ε 13,600).

Ergosta-1,4,6-*trien*-3-*one* (11).—The ketone (10) (200 mg), DDQ (145 mg), and toluene-*p*-sulphonic acid (200 mg) in dioxan (10 ml) were heated under reflux for 3 h. The product was extracted into ether, and washed with sodium thiosulphate solution, dilute sodium hydroxide, and water. It was purified by t.l.c. (silica gel) but resisted attempts at crystallisation and had $\lambda_{max.}$ (EtOH) 225, 254, and 300 nm (11,100, 10,000, and 10,800); the cholesterol analogue has $\lambda_{max.}$ 224, 258, and 300 nm (ε 10,700, 9300, and 12,900).[31]

The ketone (11) (100 mg) in acetic anhydride (5 ml) and toluene-*p*-sulphonic acid (20 mg) were heated at 100° for 5 h. Water was added and after the usual work-up, a solution in light petroleum–benzene (1 : 1) was passed through a short column of alumina to give amorphous material (12) (45 mg), which did not crystallise, $\lambda_{max.}$ (EtOH) 224 and 265 nm (23,000 and 7100); the cholesterol analogue had $\lambda_{max.}$ 226 and 264 nm (ε 31,600 and 12,300).[30]

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[29] I. M. Heilbron, T. Kennedy, F. S. Spring, and G. Swain, *J. Chem. Soc.*, 1938, 869.

[30] I. M. Heilbron and J. C. E. Simpson, *J. Chem. Soc.*, 1932, 2402.

[31] A. I. Scott, ' Interpretation of the Ultraviolet Spectra of Natural Products,' Pergamon, Oxford, 1964, pp. 410 and 421.