1117. *A Convenient Synthesis of* [9,19-$^3H_2$]*Cholecalciferol 3,5-Dinitrobenzoate and the Mechanism of the Precholecalciferol ⇌ Calciferol Reaction* *

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A convenient preparation of 19-labelled cholesterol and its conversion into cholecalciferol is described. Confirmatory evidence for the previously postulated mechanism involving an intramolecular hydrogen transfer for the precholecalciferol (II) ⇌ cholecalciferol (III) reaction is provided. The stereochemistry of the transition state (VI) for this reaction is also discussed.

The conversion of provitamin D (I) (7-dehydrocholesterol or ergosterol) into vitamin D (III) (cholecalciferol or calciferol, respectively) involves two steps. The first is induced by light and is responsible for the provitamin D (I) ⇌ previtamin D (II) reaction.[1] This is followed by a nonphotochemical reaction which results in an equilibrium mixture of previtamin D (II) and vitamin D (III), favouring the latter.[2]

* For a preliminary report see M. Akhtar and C. J. Gibbons, *Tetrahedron Letters*, 1965, 509.

[1] L. Velluz and G. Amiard, *Bull. Soc. chim. France*, 1955, **22**, 205.

[2] E. Havinga and J. L. M. A. Schlatmann, *Tetrahedron*, 1961, **16**, 146; *Rec. Trav. chim.*, 1961, **80**, 1101.

In the course of this work, which was primarily aimed at the preparation of 9,19-ditritiated cholecalciferol, we have also made observations which are relevant to the mechanism of the conversion precholecalciferol ⇌ cholecalciferol.

Recent studies on the introduction of functional groups at " nonactivated " positions in the steroid nucleus [3] have made available a variety of 18-substituted compounds.[4] One of these compounds, 19-toluene-*p*-sulphonyloxycholesteryl acetate (IV; R = Ac, X = O·SO_2·C_6H_4·CH_3-*p*),[4] on treatment with aluminium lithium hydride underwent a homoallylic rearrangement [5] to furnish 5β,19-cyclocholestan-3β-ol (V; X = α-H, β-OH), This structure is based on its n.m.r. spectrum [6] which is reported in the Experimental section. On oxidation, this compound gave the ketone (V; X = O). An analogous compound [7] in the androstane series, obtained by a different reaction sequence, has recently been converted into [19-$^3H_1$]androst-4-ene-3,17-dione. 19-Toluene-*p*-sulphonyloxy cholesteryl acetate (IV; R = Ac, X = O·SO_2·C_6H_4·CH_3-*p*) with sodium iodide in boiling ethyl methyl ketone gave the 19-iodide (IV; R = Ac, X = I) which, on reduction with zinc in the presence of tritiated water containing a trace of acid, under conditions which were carefully worked out with ordinary water, gave [19-$^3H_1$]cholesteryl acetate (IV; R = Ac, X = ^3H).[8] This, on treatment with *N*-bromosuccinimide followed by dehydrobomination and saponification, gave 7-dehydro[19-$^3H_1$]cholesterol (I).[9] The latter yielded tritiated cholecalciferol, isolated as its 3,5-dinitrobenzoate.* Radioactivity measurements revealed that no significant amount of label was lost during the conversion (I) ⟶ (III) (see Table).

Pair		Relative activity
(i)	Cholesteryl acetate	1·00
(ii)	Derived 7-dehydrocholesterol	0·96
(iii)	7-Dehydrocholesterol *	1·08
(iv)	Derived cholecalciferol *	1·00
(v)	Cholecalciferol *	1·02
(vi)	The same after being refluxed in methanol–benzene for 2 hr.	1·00
(vii)	Cholecalciferol	1·00
(viii)	Formaldehyde dimedone derivative from (vii)	0·48
(ix)	Precholecalciferol	1·05
(x)	Equilibrium mixture of cholecalciferol and precholecalciferol derived from (ix)	1·00
(xi)	Cholecalciferol	1·00
(xii)	Permanganate oxidation product of (xi) isolated as its semicarbazide	0·03

* As 3,5-dinitrobenzoate.

In another experiment, a sample of chromatographically pure tritiated precholecalciferol (II) was rearranged to an equilibrium mixture containing 80% cholecalciferol and 20% precholecalciferol; the two samples had specific activities of 1·05 and 1·0, respectively.

* For the preparation of tritiated cholecalciferol for biological purposes, we recommend its isolation from the equilibrium mixture by thin-layer chromatography rather than *via* the 3,5-dinitrobenzoate.

[3] These reactions have been reviewed by (*a*) M. Akhtar, *Adv. Photochem.*, 1964, **2**, 263; (*b*) A. L. Nussbaum and C. H. Robinson, *Tetrahedron*, 1962, **17**, 35; (*c*) T. B. Windholz and M. Windholz, *Angew. Chem. Internat. Edn.*, 1964, **3**, 353; (*d*) K. Heusler and L. Kalvoda, *ibid.*, p. 525.

[4] M. Akhtar and D. H. R. Barton, *J. Amer. Chem. Soc.*, 1964, **86**, 1528, and references cited therein.

[5] Other examples of homoallylic rearrangements involving the 19 position in the steroid nucleus are recorded: see ref. 4, and J. J. Bonet, H. Wehrli, and K. Schaffner, *Helv. Chim. Acta*, 1962, **45**, 2615; *ibid.*, 1963, **46**, 1776; J. Tadanier and W. Cole, *Tetrahedron Letters*, 1964, 1345; O. Halpern, P. Crabbé, A. D. Cross, I. Deflin, L. Cervantes, and A. Bowers, *Steroids*, 1964, **4**, 1.

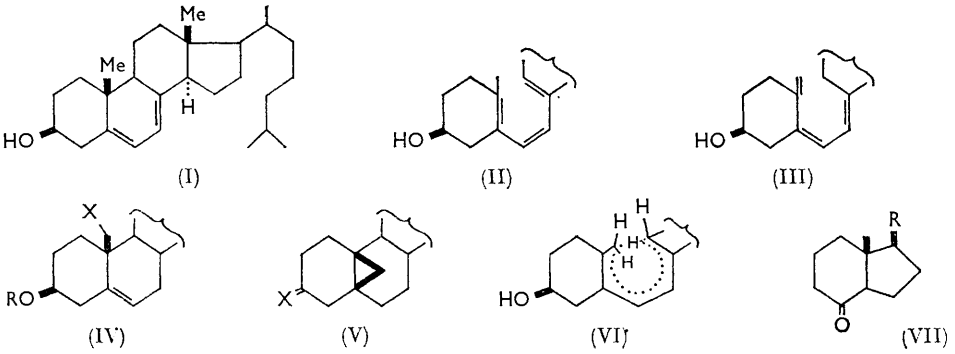
[6] We thank Mr. D. N. Henty and his staff for kindly determining the n.m.r. spectrum.

[7] S. Rakhit and M. Gut, *J. Amer. Chem. Soc.*, 1964, **86**, 1432.

[8] After this part of the work had been completed, C. Djerassi and M. A. Kielczewski (*Steroids*, 1963, **2**, 125) described a method for the introduction of deuterium into the C-19 methyl group.

[9] This compound was prepared by modifications of the method of S. Bernstein, L. J. Binovi, L. Dorfman, K. J. Sax, and Y. Subbarow, *J. Org. Chem.*, 1949, **14**, 433.

Furthermore, a sample of tritiated cholecalciferol 3,5-dinitrobenzoate after being refluxed for 2 hr. in methanol–benzene showed no loss of radioactivity (Expt. 6b). These results conclusively establish the previously postulated mechanism in which the conversion (II) ⇌ (III) takes place through an intramolecular hydrogen transfer.[10] We hoped that the distribution of radioactivity between C-19 and C-9 in cholecalciferol would throw some light on the stereochemistry of the transition state (VI) involved in the reversible reaction (II) ⇌ (III). A stereospecific delivery and migration of hydrogen atoms in the transition state (VI) would be indicated by a 2 : 1 distribution of radioactivity between C-19 and C-9, while a 1 : 1 distribution would indicate a non-stereospecific transition state in which both 9α- and 9β-hydrogen atoms are equivalent. Chromatographically purified [9,19-$^3H_2$]cholecalciferol gave, on ozonolysis, formaldehyde which was isolated as its dimedone derivative and which had 48% of the radioactivity of the cholecalciferol. Oxidation of [9,19-$^3H_2$]cholecalciferol with potassium permanganate and sulphuric acid in benzene gave the ketone (VII) isolated as its semicarbazone derivative. This latter possessed only about 3% of the radioactivity of the cholecalciferol, most of the label having been lost during oxidation. From these experiments we conclude that thermal equilibration of (II) and (III) takes place through intramolecular hydrogen transfer and that the transition state leading to this process is non-stereospecific. This is illustrated in formula (VI),



although for the sake of convenience we have shown the transition state as involving only the α-hydrogen.

Experimental

Microanalyses were performed by the Mikroanalytisches Laboratorium des Organisch-Chemischen Institutes der Universität, Vienna. Infrared spectra were determined on a Unicam S.P. 200 spectrophotometer and ultraviolet spectra on a Hilger and Watts Ultrascan recording spectrophotometer. Tritium-counting experiments were performed using an Isotope Developments Ltd. Tritium Scintillation Counter model 6012A, in conjunction with an Ecko model N529D scaler, using Nuclear Enterprises (G.B.) Ltd. scintillator No. 213. Optical rotations were measured with a Perkin-Elmer model 141 polarimeter on 0·1% solutions in chloroform.

5β,19-*Cyclocholestan*-3β-*ol*.*—19-Toluene-*p*-sulphonyloxycholesteryl acetate (IV; R = Ac, X = O·SO_2·C_6H_4·CH_3·*p*) (1·0 g.) and aluminium lithium hydride (700 mg.) were refluxed in dry ether (50 c.c.) for 2—3 hr. Working up in the usual way and crystallisation from ether–methanol gave 5β,19-*cyclocholestan*-3β-*ol* (450 mg.), m. p. 128—130°, $[\alpha]_D$ +32·5°, $\nu_{max.}$ (Nujol) 3300 cm.$^{-1}$, the n.m.r. spectrum showed no olefinic proton or 19-methyl group and had peaks at 0·91, 0·80, 0·70, (4 methyl groups), and 0·38 (cyclopropane protons) p.p.m. down-field from

* These two compounds were first prepared at the Research Institute for Medicine and Chemistry, Cambridge, Massachusetts, under the general programme for steroid synthesis.

[10] See ref. 2 and R. L. Autrey, D. H. R. Barton, A. K. Ganguly, and W. H. Reusch, *J.*, 1961, 3313; R. L. Autry, D. H. R. Barton, and W. H. Reusch, *Proc. Chem. Soc.*, 1959, 55.

tetramethylsilane as internal standard (Found: C, 84·1; H, 11·8. $C_{27}H_{46}O$ requires C, 83·9; H, 12·0%).

5β,19-*Cyclocholestan*-3-*one*.—The alcohol (V; X = α-H,β-OH) (50 mg.) was oxidised with a slight excess of chromium trioxide in acetone.[11] Addition of methanol and dilution with water gave, after the usual work-up, 5β,19-*cyclocholestan*-3-*one* (V; X = O) (25 mg.), m. p. 88—89·5°, $[\alpha]_D$ +36·5°, $\nu_{max.}$ (Nujol) 1710 cm.$^{-1}$ (Found: C, 83·9; H, 11·3. $C_{27}H_{44}O$ requires C, 84·3; H, 11·5%).

19-*Iodocholestan*-3β-*ol* 3-*Acetate* (IV; X = I, R = Ac).—19-Toluene-*p*-sulphonyloxycholesteryl acetate (IV; X = O·SO_2·C_6H_4·CH_3·*p*, R = Ac) (5·0 g.) and sodium iodide (15 g.) were refluxed in ethyl methyl ketone (200 c.c.) with efficient stirring for 6 hr. Addition of water, extraction into ether, and crystallisation from acetone–methanol gave 19-*iodocholesteryl acetate* (IV; X = I, R = Ac) (2·7 g.), m. p. 97°, $[\alpha]_D$ −56·4°, $\nu_{max.}$ (Nujol) 1725 cm.$^{-1}$ (Found: C, 62·8; H, 8·8; I, 22·8. $C_{29}H_{47}IO_2$ requires C, 62·8; H, 8·5; I, 22·9%).

[19-$^3H_1$]*Cholesteryl Acetate*.—A solution of 19-iodocholesteryl acetate (203 mg.) and toluene-*p*-sulphonic acid (80 mg.) in dry benzene (8 c.c.) and tritiated water 0·08 c.c.; 5 c/c.c.) was heated under reflux with stirring while zinc dust (2·4 g.) was added in two portions over 1·5 hr.; heating and stirring were continued for an additional 1·5 hr. Aqueous acetic acid (0·08 c.c.; 50%) was added and the previous operation continued for 10 min. Zinc was removed by filtration and the product extracted with light petroleum (b. p. 60—80°), washed with water, and dried (Na_2SO_4). The residue after evaporation was diluted with cold cholesterylacetate (600 mg.) and the product crystallised to yield [19-$^3H_1$]cholesteryl acetate (413 mg.; $4·6 \times 10^6$ c.p.s., counting efficiency 20%), m. p. 112—113°. Repeated crystallisation did not affect the specific activity. That under these conditions no random labelling occurred was demonstrated by treating tritiated cholesteryl acetate under the conditions described above using either ordinary water or tritiated water, when no change in the specific activity was observed.

7-*Dehydro*[19-$^3H_1$]*cholesterol*.—A vigorously stirred suspension of cholesteryl acetate (400 mg.) and *N*-bromosuccinimide (200 mg.) in refluxing, sodium-dried light petroleum (20 c.c.; b. p. 60—80°) was treated with a solution of bromine (0·005 c.c.) in light petroleum (5 c.c.; b. p. 60—80°) to initiate bromination. After being heated under reflux with stirring for 7 min., the mixture was cooled, washed with water, dried (Na_2SO_4), and evaporated to an oil *in vacuo* below 10°. This oil in xylene (5 c.c.) was added to a refluxing solution of *s*-collidine (0·6 c.c.) in xylene (20 c.c.) and refluxed under carbon dioxide for 8 min., cooled, washed with 0·5N-sulphuric acid, aqueous sodium hydrogen carbonate solution, and water, dried (Na_2SO_4), and evaporated to an oil *in vacuo* as rapidly as possible. Residual xylene was removed by adding ethanol (5 c.c.) and re-evaporating. Two such ethanol treatments gave a solid which was taken in acetone (4 c.c.) and left at 0° for 12 hr. to yield 7-dehydrocholesteryl acetate (114 mg., 28·5%), m. p. 126°, $\lambda_{max.}$ (in ether) 272, 282, and 294 mμ (ε 10,100, 10,600, and 6000).[9] This acetate (100 mg.), in benzene (0·4 c.c.) and methanol (3 c.c.), was treated with methanolic potassium hydroxide (0·5 c.c.; 2N) and the reaction mixture left at room temperature for 10 min. to yield after usual work-up 7-dehydrocholesterol (79 mg., 88%), m. p. 139—141°, $\lambda_{max.}$ (in ether) 272, 282, and 294 mμ (ε 10,500, 11,500, and 6500).[9]

Similarly, [19-$^3H_1$]cholesteryl acetate (1660 μc/mole) gave 7-dehydro[19-$^3H_1$]cholesteryl acetate (1578 μc/mole).

For a more active preparation, [19-$^3H_1$]cholesteryl acetate (400 mg.; 9885 counts/sec./mg.) was processed as above to yield, after dilution with cold 7-dehydrocholesteryl acetate (100 mg.) prior to crystallisation, 7-dehydro[19-$^3H_1$]cholesterol of activity 4130 counts/mg./sec.

Photolysis of 7-*Dehydro*[19-$^3H_1$]*cholesterol and Related Experiments*.—(*a*) A solution of 7-dehydro[19-$^3H_1$]cholesterol (200 mg.) (dinitrobenzoate, m. p. 205°, 997 counts/mg./min.) in dry benzene (80 c.c.) was irradiated for 8 min. by means of a 125-w high-pressure mercury arc lamp (A.E.I. Co.) placed inside a water-cooled quartz immersion well. Agitation of the solution, which was cooled in an ice-bath, was maintained by a stream of carbon dioxide. The photolysis mixture was evaporated *in vacuo* at 5—10°. The residue in ether (1 c.c.) was spotted on five silica gel (Merck, Kieselgel HF_{254}) plates (20 × 9 cm.; 2 mm. thick layer) which were developed twice in chloroform and then viewed in ultraviolet light. The least polar bands gave, after extraction in ether, precholecalciferol, (66—75 mg., estimated spectrophotometrically), $\lambda_{max.}$ (in ether) 261 mμ. This was dissolved in benzene (10 c.c.) and methanol (1 c.c.) and refluxed

[11] K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J.*, 1946, 39.

for 2 hr. Evaporation of the solvent yielded an oil containing an equilibrium mixture of cholecalciferol (80%) and precholecalciferol (20%). This was treated with 3,5-dinitrobenzoyl chloride (300 mg.) in pyridine (2 c.c.) and benzene (3 c.c.) for 1 hr. to yield, after the usual work-up, [9,19-$^3H_2$]cholecalciferol 3,5-dinitrobenzoate [12] (20 mg., 923 counts/mg./min.), m. p. 126—128°.

(*b*) A solution of [9,19-$^3H_2$]cholecalciferol 3,5-dinitrobenzoate (30 mg., 245 counts/mg./min.) in methanol (2 c.c.) and benzene (8 c.c.) was refluxed for 2 hr. Evaporation of the solvent and crystallisation from acetone–methanol gave the starting material (20 mg., 240 counts/mg./min.).

(*c*) A solution of chromatographically homogeneous precholecalciferol [prepared as in (*a*)] in ether (10 c.c.) was divided into two equal portions. One portion was rearranged to an equilibrium mixture of cholecalciferol (80%) and precholecalciferol (20%) and lyophilised for 18 hr. The other portion was lyophilised without rearrangement. These samples had radioactivities of 3570 and 3740 counts/min., respectively.

(*d*) A solution of 7-dehydrocholesterol (200 mg., 355 μc/mole) in benzene (100 c.c.) was irradiated as described above. After being refluxed for 2 hr., the benzene was removed *in vacuo* and the residue ozonised in acetic acid (10 c.c.) and acetic anhydride (30 c.c.). Formaldehyde was isolated as the dimedone derivative (48 mg., 47%; 147 μc/mole), m. p. 179—181°. Refluxing the photolysis mixture for 1 and for 5 hr. did not alter the radioactivity of the dimedone derivative.

(*e*) A solution of chromatographically purified precholecalciferol [prepared as in (*a*)] was rearranged by refluxing in benzene for 2 hr. and the reaction mixture separated by preparative thin-layer chromatography, as in (*a*), into the less polar precholecalciferol and the more polar cholecalciferol bands. Elution of the latter with ether gave tritiated cholecalciferol of high purity. Cholecalciferol (115 mg.; 285 counts/min./μmole) was ozonised as in (*d*) and gave formaldehyde, isolated as its dimedone derivative (54 mg.; 61%; 137 counts/min./μmole), m. p. 180°.

(*f*) Chromatographically pure cholecalciferol [prepared as in (*e*)] (230 mg., 285 counts/min./μmole) in benzene (90 c.c.) was oxidised with potassium permanganate (1·4 g.) and sulphuric acid (0·3 c.c.) in water (40 c.c.) at 0° for 3 hr.[13] The organic layer yielded the ketone (VII), isolated as its semicarbazone [14] (59 mg., 30%; 8590 counts/min./mmole), m. p. 208—211°.

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[12] A. Windaus, F. Schenk, and F. von Werder, *Z. physiol.*, 1936, **241**, 100.
[13] A. Windaus and W. Grundman, *Annalen*, 1936, **524**, 295.
[14] A. Windaus, M. Deppe, and W. Wunderlich, *Annalen*, 1937, **535**, 118.