102. *The Catalytic Deuteration of Organic Compounds. Part II.* The Deuteration of Cholesterol and Ergosterol.*

By J. Bell and S. J. Thomson.

Deuteriocholesterol and deuterioergosterol have been prepared under comparable experimental conditions by Bloch and Rittenberg's method, *i.e.*, a platinum-catalysed exchange reaction between the sterols and deuterium oxide. A reaction involving the migration of double bonds has been shown to occur, by the isolation of deuteriocholest-4-en-3-one and deuterioergosterol B_1, in cholesterol and ergosterol deuterations respectively. The role of the solvent, acetic acid and deuterium oxide, has been studied, and the possibility of the deuteration of the acetic acid in the methyl group being an essential preliminary to sterol deuteration has been eliminated. The deuteration of cholesterol by use of an acid other than acetic together with a platinum catalyst was unsuccessful.

Deuteriocholesterol and deuterioergosterol have been prepared under comparable experimental conditions by Bloch and Rittenbergs's method (*J. Biol. Chem.*, 1943, **149**, 505), the sterols being shaken for three days at 123—133° in sealed evacuated tubes containing heavy water, acetic acid, and Adams's platinum catalyst. By this means deuterium was introduced into the sterols and the methyl group of the acetic acid solvent. In accordance with Bloch and Rittenberg's work considerable destruction of the sterols was observed.

The annexed Table shows the extent of these deuteration reactions for various quantities of Adams's catalyst.

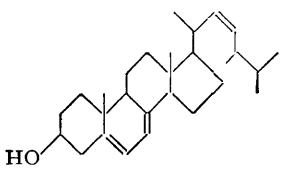
Deuteration of ergosterol and cholesterol.

	Cholesterol			Ergosterol		
Experiment	1A	2A	3A	1B	2B	3B
G. of Pt per g. of sterol	0·1218	0·2352	0·3582	0·1263	0·2343	0·3631
Atom % deuterium in sterol	1·36	1·48	2·12	0·93	2·66	2·79
Atom % deuterium in methyl group of acetic acid solvent	3·06	3·65	4·12	2·30	3·43	7·83

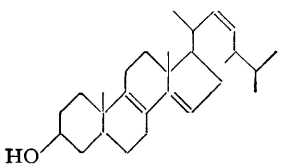
Consideration of these reactions in the light of the information gained in Part I,* suggested that those parts of the sterol molecule containing only saturated carbon chains would contain little deuterium, whereas greater deuteration would occur in the vicinity of the hydroxyl group and the double bonds.

The increased deuteration of ergosterol as shown in Experiments 2 and 3 might be attributed to the conjugated double bonds of ring B and the double bond of the side chain.

It had been observed in Part I that considerable exchange had occurred together with a disproportionation reaction. This could be explained by assuming that, under the influence of the catalyst, hydrogen was removed from some molecules and deuterium or hydrogen from the solvent added to others.



Ergosterol



Ergosterol B_1

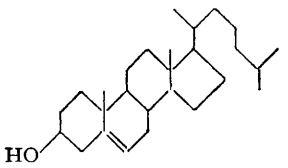
Some such process might be operative in the deuteration of sterols containing double bonds. A reaction similar to the disproportionation would be the removal of hydrogen from a ring of the sterol molecule and the addition of hydrogen or deuterium to the double bond position, the removal and addition reactions taking place in either this or the reverse

* Part I, preceding paper.

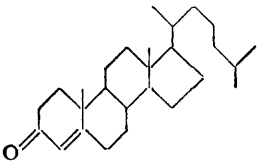
order. This would result in the migration of double bonds in the sterol rings and the consequent production of isomers containing relatively large amounts of deuterium.

The isolation of deuterioergosteryl B_1 acetate on treatment of ergosterol with deuterium oxide, acetic acid, and a platinum-black-on-asbestos catalyst showed that double-bond migration had occurred. Furthermore, the sterol portion of the ester contained 3·68 atom % of deuterium, probably in the 5, 6, and 7 positions.

Similar treatment of cholesterol yielded cholest-4-en-3-one containing 0·21 atom % of deuterium. Again double-bond migration had occurred together with deuteration. In this case the parent sterol was isolated and was found to contain 0·12 atom % of deuterium.



Cholesterol



Cholest-4-en-3-one

This type of side reaction may have accounted for some of the destruction of the sterol during deuteration recorded by Bloch and Rittenberg and earlier in this paper.

Since deuteriocholest-4-en-3-one was produced during the deuteration of cholesterol it seemed not unreasonable to examine positions 3 and 4 of the deuteriocholesterol for deuterium atoms. This was accomplished by converting a sample of the deuteriocholesterol into cholest-4-en-3-one, thereby removing some of the deuterium or hydrogen atoms from positions 3 and 4. The atom % of deuterium in both cholesterol and the resultant cholest-4-en-3-one was 0·12. It must be concluded that the deuteriocholesterol did not contain deuterium in position 3 or 4.

During the deuteration of ergosterol and cholesterol the deuterio-isomerisation products were isolated in small yields. Such deuterations by isomerisation in heavy water might, however, be extended to the preparation of deuterio-organic compounds normally produced in reasonable yields by isomerisation reactions.

From the results shown in Part I, Table 1, and in Part II (p. 576), it will be seen that in all cases deuterium was introduced into the methyl group of the acetic acid, in conformity with the observation made in the course of this work that relatively large amounts of deuterium could be introduced into acetic acid by similar treatment with heavy water in presence of catalysts.

The function of the acetic acid appeared to be not only that of solvent, since an attempted deuteration of cholesterol with heavy water, a platinum catalyst, and dilute sulphuric acid in alcohol failed. Only very small quantities of deuterium were introduced into the sterol product by treatment of ergosterol with anhydrous deuterioacetic acid (with deuterium atoms in the methyl group only), indicating that the production of the deuterio-acid is unlikely to be an essential preliminary to the deuteration of the sterol.

Experimental

Preparation of Deuterio-cholesterol and -ergosterol.—Acetic acid, heavy water, and platinum oxide (Adams's catalyst) in the quantities shown in the Table below were weighed into 6″ × ½″ tubes. Hydrogen was passed into the suspension for 90 minutes, followed by nitrogen for 60 minutes to remove as much of the hydrogen as possible. The weighed quantities of the sterols were added and the contents of the tube frozen. After evacuation and sealing, the tubes were shaken for 3 days at 123—133°.

Expt.	Sterol (g.)	D_2O (g.)	H_2O (g.)	AcOH (g.)	PtO_2,H_2O (g.)
i, E_1	0·7975	0·6650	0·1515	2·8060	0·1270
C_1	0·7945	0·6490	0·2090	2·7850	0·1220
ii, E_2	0·7850	0·6975	0·2075	2·7460	0·2320
C_2	0·7925	0·6680	0·2045	2·7345	0·2355
iii, E_3	0·7870	0·6480	0·2040	2·7450	0·3600
C_3	0·7970	0·6520	0·2055	2·7180	0·3600

Recovery of the Sterols.—The acetic acid and water were recovered by vacuum-distillation, the residues from the distillation were warmed with ether, and the catalyst was filtered off. The filtrate was evaporated to dryness, and the steryl acetates were hydrolysed with alcoholic potassium hydroxide. The cholesteryl acetate was refluxed for 40 minutes with 40 ml. of 2% solution, and the ergosteryl acetate with 60 ml. of 5% solution. Boiling under reduced pressure and slow evaporation removed the alcohol. The residues were extracted with water and then with ether. The ethereal extracts on evaporation yielded gums which gave crystalline sterols from alcohol, benzene–alcohol, and acetone. In later experiments this process was superseded by chromatographic separation on alumina.

Cholesterol and Ergosterol Chromatography.—The columns were prepared from alumina suspensions in benzene and the sterols added in light petroleum (b. p. 60—80°) solution. The eluants were light petroleum and benzene. The sterols produced were crystallised from dry alcohol.

M. p.s (in a vacuum), mixed m. p.s with the parent sterols, and yields were as follows :

Ergosterol : Expt. E1, m. p. 164·3°, mixed m. p. 163·5° (with ergosterol, m. p. 163·5°), 0·067 g.; Expt. E2, m. p. 150—151°, mixed m. p. 158—161°, 0·090 g.; Expt. E3, m. p. 160°, mixed m. p. 163°, 0·029 g.

Cholesterol : Expt. C1, m. p. 145·5°, mixed m. p. 145·5° (with cholesterol, m. p. 146—147°), 0·090 g.; Expt. C2, m. p. 142°, mixed m. p. 144—146°, 0·140 g.; Expt. C3, m. p. 145°, mixed m. p. 146—147°, 0·099 g.

Recovery of Acetic Acid Solvent.—The distillate recovered after the exchange reaction consisted of water and acetic acid. The acid was converted into silver acetate by the method described in Part I.

Starting Materials.—Ergosterol. Commercial ergosterol was purified by Bills and Cox's method (*J. Biol. Chem.*, 1929, **84**, 455) using 2 : 1 alcohol–benzene for crystallisations. After purification, the sterol melted at 163·5° (in a vacuum), and had $[\alpha]_{Hg}^{20}$ −160·2° (*c*, 1% in chloroform).

Cholesterol. Alcohol extraction of gallstones and repeated crystallisations from alcohol gave a product, m. p. 146—147°.

Acetic Acid. This was purified by refluxing it over chromium trioxide, distillation, and crystallisation.

Deuterium oxide. 99·6% by weight, " Norsk Hydro."

Water. Ordinary distilled water was redistilled four times, the first two distillations being from potassium permanganate.

Investigation of the Products in an Attempted Deuteration of Ergosterol.—A platinum-black-on-asbestos catalyst was prepared by formaldehyde reduction of a platinum salt solution (Vogel, " Textbook of Practical Organic Chemistry," Longmans, 1948, p. 824, Method A). Ergosterol {4·804 g.; m. p. 163·5—164·8° (in a vacuum), $[\alpha]_{Hg}^{18}$ −163·5°}, acetic acid (17·381 g.), water (1·300 g.), deuterium oxide (4·153 g.), and catalyst (0·810 g.) were placed in a " Pyrex " reaction vessel which was then cooled in a freezing mixture, evacuated, sealed, and shaken for 3 days at 124—135°. The tube was cooled and the volatile components removed by vacuum-distillation : 22·291 g. were recovered, leaving 0·543 g. as residue, probably as steryl acetate. The residues were dissolved in chloroform, the catalyst was filtered off, and the filtrate evaporated to a gum and dissolved in benzene. An alumina chromatograph column was moistened with light petroleum (b. p. 60—80°). The benzene solution was added and elution performed with light petroleum (b. p. 60—80°), benzene, chloroform, alcohol, and acetone. Each portion of the eluant was evaporated and the residues obtained were boiled with charcoal in alcohol. Many attempts to crystallise these products were made, using a great variety of methods and solvents. Finally " fraction 10 " yielded crystals from alcohol when the solution (10 ml.) was poured into a vessel containing 0·2 ml. of water. The white crystals obtained, m. p. 131°, crystallised from benzene–alcohol (1 : 4) as small elongated plates, m. p. 135—136·5°, $[\alpha]_{D}^{18}$ −50·9° in chloroform : the yield of pure material was 0·05 g., $\lambda_{max.}^{CHCl_3}$ 248 mμ (Found : C, 81·9; H, 10·3. Calc. for $C_{30}H_{46}O_2$: C, 82·1; H, 10·6%).

Examination of the other fractions by Windaus's method (*Annalen*, 1931, **488**, 91) suggested that ergosterols B_2 and B_3 were absent. In a second deuteration, ergosterol (5·433 g.; m. p. 163·5° (*in vacuo*), $[\alpha]_{Hg}^{19}$ −166·4° in chloroform), acetic acid (17·230 g.), water (1·295 g.), deuterium oxide (4·288 g.), and catalyst (0·810 g.) were shaken as before for 3 days at 125—129°; 21·500 g. of solvent were recovered. The solids remaining were dissolved in hot alcohol, charcoal was added, and the catalyst and charcoal were filtered off. The filtrate was placed in a refrigerator and a crop of crystals collected, having m. p. 128—131°. Two further crops, collected similarly by

reduction of the volume of alcohol, had m. p. 125° and 124·5° respectively. These three samples were combined (1·15 g.) and many crystallisations from alcohol yielded a product (0·50 g.), m. p. 139·5—141°, $[\alpha]_D^{18\cdot5}$ −56·1° in chloroform, $\lambda_{max.}^{ether}$ 249 mμ (Found: C, 82·3; H, 10·3%) (cf. Windaus, m. p. 142°, $[\alpha]_D^{19}$ −53·6°, $\lambda_{max.}$ for ergosterol B_1 248 mμ in ether).

Deuteration of Cholesterol.—Starting materials. The water, acetic acid, and platinum catalyst were from stocks prepared for the previous experiments. The deuterium oxide (" Norsk Hydro ") was 99·6% by weight.

Cholesterol (10·580 g.), acetic acid (33·832 g.), water (2·675 g.), deuterium oxide (8·960 g.), and catalyst (1·447 g.) were shaken as before for 3 days at 125—129°. The acetic acid and water were recovered by vacuum-distillation, and the acid was converted into silver acetate. The residues were dissolved in hot alcohol, and the catalyst was filtered off. An alumina column for chromatographic analysis was prepared and moistened with carbon tetrachloride. The sterol products were added in a solution of the same solvent. Elution was by carbon tetrachloride, and by 0·1, 0·2, 0·5, 1, 2, 5, 10, 50, and 100% alcohol in carbon tetrachloride.

The twenty-five fractions so obtained were evaporated and attempts made to crystallise the residues. Gums or oils were obtained from all fractions with the exception of the following:

Fraction 4: Crystallisations from methyl alcohol and acetone mixtures, followed by ethyl alcohol, yielded cholesteryl acetate (0·21 g.), m. p. 114·5° $[\alpha]_D^{19}$ −40·5° in chloroform.

Fractions 7—12: Methyl alcohol crystallisations yielded cholest-4-en-3-one (0·3 g.), m. p. 80°, $[\alpha]_D^{19}$ + 86·7° in chloroform, $\lambda_{max.}^{ether}$ 234 mμ; it gave a semicarbazone, m. p. 231° (decomp.) (Found: C, 76·1; H, 10·5; N, 9·7. Calc. for $C_{28}H_{47}ON_3$: C, 76·1; H, 10·7; N, 9·5%).

Fraction 14: Ethyl alcohol crystallisations yielded cholesterol (0·55 g.), m. p. 147·5°, $[\alpha]_D^{20}$ −40·0° in chloroform.

Location of Deuterium Atoms in Deuteriocholesterol. Conversion of Deuteriocholesterol into Cholest-4-en-3-one.—Bromination (Windaus, *Ber.*, 1906, **39**, 518). Deuteriocholesterol (0·464 g.) was dissolved in ether (4·6 ml.), and a solution of bromine in acetic acid added (2·32 ml. of a solution of 2·032 g. of bromine in 21·135 g. of glacial acetic acid). The mixed liquids set to a solid yellowish mass almost at once and this was stirred with diluted acetic acid (3 : 1 acetic acid : water), filtered, washed with water, and dried in a vacuum.

Oxidation of cholesterol dibromide (Ruzicka *et al.*, *Helv. Chim. Acta*, 1934, **17**, 1407). The dibromide (0·65 g.) was dissolved in benzene (6·5 ml.), and a solution of chromium trioxide in aqueous acetic acid added (6·5 ml. of a solution of 1·2 g. of chromium trioxide in 12 ml. of water and 24 ml. of acetic acid). The liquids were shaken together for 6 hours at room temperature, and the benzene layer was separated and washed with water until neutral to litmus. After being dried (Na_2SO_4), the benzene was evaporated off, leaving a residue of 5 : 6-dibromocholestan-3-one.

Debromination (Butenandt and Schmidt-Thomé, *Ber.*, 1936, **69**, 882). The cholestanone dibromide (0·3 g.) was dissolved in a mixture of ethyl alcohol (10 ml.) and methyl alcohol (10 ml.), and zinc dust (0·3 g.) was added with dilute sulphuric acid (0·8 ml. of 4N-acid). The suspension was boiled for 10 minutes and filtered, and the alcohol removed from the filtrate under vacuum. The residue was crystallised four times from methyl alcohol. The product was cholest-4-en-3-one (0·11 g.), m. p. 79·5°.

Attempted Deuteration of Cholesterol with Deuterium Oxide, a Platinum Catalyst, and Dilute Sulphuric Acid in Alcohol.—Adams's catalyst (0·393 g.) was weighed into alcohol, and hydrogen passed in for 1 hour to reduce the catalyst to its active form. Nitrogen, to remove as much hydrogen as possible, was passed through the suspension for 2 hours. The weight of alcohol was made up to 9·544 g., and deuterium oxide (2·211 g.; 99·6% by weight, " Norsk Hydro "), sulphuric acid (1·090 g. of 4N-acid), and cholesterol (2·547 g. of a sample, m. p. 148·5—149°, $[\alpha]_D^{20}$ −39·5°) were added. This mixture was refluxed on a steam-bath for 26 hours. Hot alcohol was added and the catalyst filtered off. Crystals separated from the filtrate on cooling. Crystallised from alcohol, these (1·2 g.) had m. p. 148°.

Deuteration of Ergosterol with Glacial Deuterioacetic Acid and a Platinum Catalyst.—Preparation of the acid. Acetic acid (1·04 g.), deuterium oxide (0·55 g.; 99·8% by weight), and platinum-black-on-asbestos catalyst (0·05 g.) were shaken for 3 days at 123—133°, in a sealed evacuated tube. The acid was isolated as the silver salt. Silver deuterioacetate (12·3 g.) was refluxed for 20 minutes with syrupy phosphoric acid (16 ml.). The mixture was then distilled and the acetic acid boiling at 115—116° collected. 4 Ml. of ordinary acetic acid were added to the 2 ml. of deuterioacetic acid; the mixture had m.p. 7°, *i.e.*, 93·4% acetic acid (6·6% H_2O). This mixture was dehydrated by refluxing it with triacetyl borate (4·2 g.) (Pictet and Geleznoff, *Ber.*, 1903, **36**, 2219) for 4 hours. The acid produced by distillation melted at 16°. This treat-

ment with triacetyl borate was repeated twice more, 0·7 g. being used each time. The product melted at 16·9°. The volume of glacial deuterioacetic acid was 7·5 ml. A few ml. of this acid were converted into silver acetate for deuterium estimation.

Deuteration. Glacial deuterioacetic acid (3·165 g.), a platinum-black-on-asbestos catalyst (0·622 g.), and ergosterol (0·792 g. of a sample, m. p. 163·5°, $[\alpha]_{Hg}^{20}$ −160·2° in chloroform) were shaken for 3 days at 123—133°. The acid was distilled off under reduced pressure and converted into silver acetate. The sterol residue was dissolved in ether, and the catalyst filtered off. The residue obtained on evaporation of the ether was hydrolysed with alcoholic potassium hydroxide (2·5 g. in 2 ml. of water and 50 ml. of alcohol). The alcohol was removed under a vacuum and the residue extracted with water. The sterol portion was then dried and dissolved in light petroleum (b. p. 40—60°; 80 ml.)–benzene (10 ml.). This solution was then added to an alumina chromatograph column moistened with benzene. Elution was with light petroleum (b. p. 40—60°), followed by benzene and benzene–alcohol (1 : 4 by volume).

Oils and gums were obtained from the chromatograph with some white solid. This was crystallised from alcohol many times and finally had m. p. 142° in a vacuum. Further crystallisation from benzene–alcohol (1 : 2 by volume) yielded 0·089 g. of a product, m. p. 145°, $[\alpha]_D$ −63·5° in chloroform, probably impure ergosterol B_1 mixed with the original sterol (ergosterol B_1, m. p. 148°, $[\alpha]_D$ −40°).

Deuterium contents. Atom % deuterium in methyl group of acetic acid before deuteration, 1·65. After deuteration, 1·63. Atom % deuterium in sterol, 0·17.

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