Preparation of 5α-[3β-^{2}H]Cholestan-3α-ol by Isomerization Reactions

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The preparation of 5α-[3β-^{2}H]cholestan-3α-ol by isomerization of the easily accessible 5α-[3α-^{2}H]cholestan-3β-ol was investigated. Isomerization of the mesylate in collidine–water or treatment of the 3β-ol with benzoic acid in the presence of triphenylphosphine and diethyl azodicarboxylate yielded the 3α-isomer with no loss of deuterium from C-3.

LABELLING of hydroxy-steroids with deuterium at the hydroxylated carbon atoms is most easily achieved by reduction of the corresponding oxo-steroids with sodium borodeuteride or lithium aluminium deuteride. However, this method cannot be used to obtain labelled 3α-hydroxy-5α-steroids since these reductions give almost exclusively the equatorial 3β-isomer.[1] The present investigation was carried out to determine whether epimerization reactions[2,3] could be used to prepare 3α-hydroxy-5α-[3β-^{2}H]steroids of high isotopic purity.

Four different experiments were performed, and the results are summarized in the Table. Insignificant loss of deuterium was observed during isomerization of the labelled 3β-mesyloxy-steroid in collidine–water, yielding a mixture of 5α-cholestan-3α-ol (50%), 5α-cholest-2-ene (40%), and small amounts of 5α-cholestan-3β-ol. Similarly the reaction of the labelled 3β-hydroxy-steroid with benzoic acid in the presence of triphenylphosphine and diethyl azodicarboxylate[3] gave 5α-cholestan-3α-yl benzoate in 90% yield with little deuterium loss. There was no detectable incorporation of deuterium into an unlabelled steroid during isomerization in deuterium oxide. Little, if any, randomization of deuterium took place during inversion of configuration.

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[1] D. M. S. Wheeler and M. M. Wheeler, in ' Organic Reactions in Steroid Chemistry,' vol. 1, eds. J. Fried and J. A. Edwards, Van Nostrand Reinhold, New York, 1972, p. 61.

[2] L. F. Fieser and M. Fieser, in ' Steroids,' Reinhold, New York, 1959, p. 324.

[3] A. K. Bose, B. Lal, W. A. Hoffman, and M. S. Manhas, *Tetrahedron Letters,* 1973, 1619 (see references to the original method by Mitsunobi and Yamasaka in this paper).

These methods of inversion of readily available 3β-hydroxy-5α-[3α-^{2}H]steroids provide simple and practical means of preparation of 3α-hydroxy-5α-[3β-^{2}H]steroids with high retention of label.

EXPERIMENTAL

Gas chromatography–mass spectrometry (g.l.c.–m.s.) was carried out with an LKB 9000 instrument (column of 1·5% SE-30 on Chromosorb W HP). Multiple spectra were recorded on magnetic tape and average isotope content was determined by comparisons with the unlabelled compound using an IBM 1800 computer.[4]

Isomerization of 5α-[3α-^{2}H]*Cholestan-3β-yl Mesylate.*—A solution of the mesylate [5] (20 mg) in collidine (0·85 ml) and water (0·15 ml) was refluxed for 24 h, poured on ice, and extracted with ether. The extract was washed with M-hydrochloric acid and water, dried, and evaporated. The trimethylsilyl ether, prepared in the usual way, was analysed by g.l.c.–m.s. The sample was also analysed by t.l.c. on silica gel (chloroform as eluant). Authentic samples of 5α-cholestan-3β- and -3α-ols and 5α-cholest-2-ene served as reference compounds.

Isomerization of 5α-[3α-^{2}H]*Cholestan-3β-ol.*[3]—To a solution of the steroid (10 mg), triphenylphosphine (14 mg) and benzoic acid (6·3 mg) in dry tetrahydrofuran (0·5 ml) diethyl azodicarboxylate (4·2 mg) in tetrahydrofuran (0·3 ml) was added with stirring and with exclusion of moisture. After 24 h at room temperature, the solvent was removed under vacuum. The residue, in ether, was washed several times with aqueous 20% potassium hydroxide, water, and dilute acid, dried, and evaporated. The product was dissolved in dry tetrahydrofuran, lithium aluminium hydride (10 mg) was added, and the mixture was refluxed for 1 h, acidified, diluted with ether, washed, and evaporated. A sample of the product was converted into the trimethylsilyl ether and was analysed by g.l.c.–m.s.

Oxidation of the Isomerization Products.—To a solution of 5α-[3α-^{2}H]cholestan-3β-ol (15 mg) in dry methylene chloride (2 ml) was added chromium trioxide–pyridine complex [6] (60 mg), and the mixture was stirred at room temperature overnight. The solvent was evaporated off in a stream of dry nitrogen and the residue was dissolved in dry pyridine (1 ml). Methoxyamine hydrochloride (15 mg) was then added and the mixture was left for 24 h with exclusion of moisture. The solvent was evaporated off in a stream of dry nitrogen and the residue was dissolved in ether. The solution was washed with water, dried (Na_2SO_4), and evaporated.

Formation of deuteriated and unlabelled 5α-cholestan-3α-ol under various conditions of epimerization of unlabelled and 3α-deuteriated 5α-cholestan-3β-ol

	Deuterium content and reaction conditions							
	[3α-^{2}H]-3β-yl mesylate + H_2O		[3α-^{2}H]-3β-yl mesylate + ^2H_2O		[3α-^{1}H]-3β-yl mesylate + ^2H_2O		[3α-^{2}H]-3β-ol + Ph_3P + $PhCO_2H$ + $(NCO_2Et)_2$	
Starting material and products	$^1H_1$	$^2H_1$	$^1H_1$	$^2H_1$	$^1H_1$	$^2H_1$	$^1H_1$	$^2H_1$
5α-Cholestan-3β-ol [a]	2·1	97·9	2·1	97·9	100		2·1	97·9
5α-Cholestan-3α-ol [b]	3·4	96·6	3·6	96·4	99·9	0·1	3·3	96·7
5α-Cholestan-3-one [c]	96·8	3·2	96·5	3·5	99·7	0·3	98·3	1·7

[a] Starting material. [b] Epimerization product. [c] Formed by oxidation of epimerization product; converted into the *O*-methyloxime for estimation of ^{2}H content.

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[6] J. C. Collins, W. W. Hess, and F. J. Frank, *Tetrahedron Letters*, 1968, 3363.