Steroids and Walden Inversion. Part XXII. The Configuration of Cholesterylamine.*

By J. H. Pierce, C. W. Shoppee, and G. H. R. Summers.

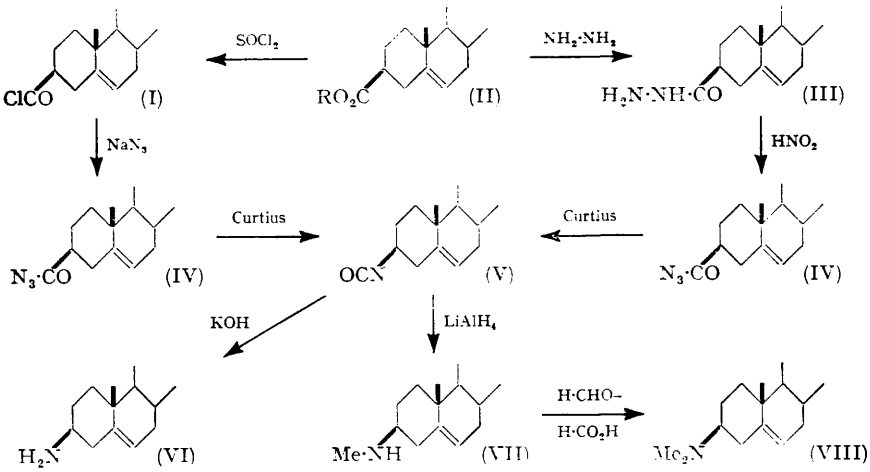
[Reprint Order No. 5739.]

Cholest-5-ene-3β-carboxylic acid (Marker's acid) has been converted into the azide by way of the chloride and by treatment of the hydrazide with nitrous acid. The azide undergoes the Curtius rearrangement to give the *iso*cyanate, which by alkaline hydrolysis affords 3β-aminocholest-5-ene; alternatively, reduction of the *iso*cyanate with lithium aluminium hydride yields 3β-methylaminocholest-5-ene. The configurations of these bases follow from the known configuration of Marker's acid, and the preservation of configuration in the migrating group in the Curtius rearrangement.

In connection with concurrent work it became necessary to establish the configuration at $C_{(3)}$ of cholesterylamine. This primary base, m. p. 98°, was obtained from cholesteryl chloride by treatment with ethanolic ammonia at 100° in the presence of ammonium iodide by Windaus and Adamla (*Ber.*, 1911, **44**, 3051). Subsequently, it was prepared (m. p. 94°, $[\alpha]_D$ −26°) from cholesteryl toluene-*p*-sulphonate by treatment with liquid ammonia at 98° by Julian, Cole, and Magnani (*J. Amer. Chem. Soc.*, 1948, **70**, 1834), and (m. p. 90—91°) by Howarth, McKenna, and Powell (*J.*, 1953, 1110); the British authors

* Part XXI, preceding paper.

also treated cholesteryl toluene-*p*-sulphonate with monomethylamine and with dimethylamine and obtained a 3-methylaminocholest-5-ene, m. p. 113°, and a dimethylamino-analogue, m. p. 152°, $[\alpha]_D$ −32°. We have prepared 3β-aminocholest-5-ene (VI) by the following method :



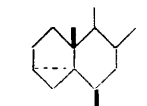
Cholest-5-ene-3β-carboxylic acid (Marker's acid) (II; R = H), in which the β-configuration of the carboxyl group is established (Roberts, Shoppee, and Stephenson, *J.*, 1954, 2705; cf. Corey and Sneen, *J. Amer. Chem. Soc.*, 1953, **75**, 6234), was converted into the chloride (I) which by treatment with dry sodium azide in anhydrous acetone–dioxan gave the azide (IV). Alternatively, the methyl ester (II; R = Me) was treated with hydrazine to yield the hydrazide (III), which by treatment with nitrous acid in benzene–acetic acid gave the azide (IV).

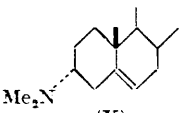
The Curtius rearrangement of azides is an intramolecular change in which configuration in the migrating group is preserved, since Kenyon and Young (*J.*, 1941, 263) have converted (+)-hydratropazide into (−)-1-phenylethylamine of over 99% optical purity, whilst Jones and Wallis (*J. Amer. Chem. Soc.*, 1926, **48**, 169) have transformed (+)-α-benzylpropionazide into (−)-1-benzylethylamine which Kenyon, Phillips, and Pitman (*J.*, 1935, 1072) showed to possess the same configuration. The azide (IV) by the Curtius rearrangement furnished the *iso*cyanate (V), m. p. 90—96°; attempts to purify the crude *iso*cyanate by recrystallisation from inert solvents or from acetone–methanol led to the production of high-melting compounds (*s*-dicholest-5-en-3β-ylurea?) and the urethane respectively.

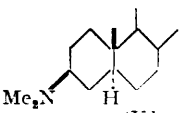
Hydrolysis of the crude crystalline *iso*cyanate (V), prepared by either procedure, with methanolic potassium hydroxide gave after chromatography 3β-aminocholest-5-ene (VI), m. p. 94°, $[\alpha]_D$ −26°; a second compound, m. p. 139—140°, $[\alpha]_D$ −37°, after crystallisation from acetone, was also isolated and was regarded as a polymorph of the base (VI), because by brief treatment with acetic anhydride it was converted into the acetyl derivative of the base (VI), m. p. 238—239°, $[\alpha]_D$ −41° [Windaus and Adamla (*loc. cit.*) report m. p. 238—242°], which by hydrogenation with platinum oxide in acetic acid gave 3β-acetamidocholestane, m. p. 242—243°, $[\alpha]_D$ +11·5°, identical with a sample, m. p. 245°, $[\alpha]_D$ +12°, prepared by reduction of cholestan-3-one oxime with sodium–ethanol according to the procedure of Howarth and Dodgson (*J.*, 1952, 67). Professor R. D. Howarth informed us on September 11th, that the Sheffield group had encountered a compound, m. p. 140°, $[\alpha]_D$ −33°, which is identical with our supposed polymorph; Professor Howarth has further most kindly informed us in a letter of September 30th that the compound is, in fact, the *iso*propylidene derivative of the base (VI), from which it is indistinguishable by elementary analysis.

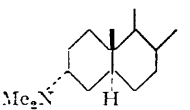
Reduction of the crude crystalline *iso*cyanate (V) with lithium aluminium hydride gave 3β-aminocholest-5-ene (VII), m. p. 113°, $[\alpha]_D$ −37°, which was identical with a specimen prepared from cholesteryl toluene-*p*-sulphonate by treatment with ethanolic methyl-

amine at 100° according to the directions of Haworth, McKenna, and Powell (*loc. cit.*), and which by methylation with formaldehyde–formic acid gave 3β-dimethylaminocholest-5-ene (VIII), m. p. 151°, $[\alpha]_D$ −32° {Haworth, McKenna, and Powell, *loc. cit.*, give m. p. 151°, $[\alpha]_D$ −31°, whilst Sorm, Labler, and Czerny (*Chem. Listy*, 1953, **47**, 418) give m. p. 151°, $[\alpha]_D$ −32°}. Solvolysis of cholesteryl toluene-*p*-sulphonate with methylamine also afforded 6β-methylamino-3 : 5-*cyclo*cholestane (IX), m. p. 52—54°, $[\alpha]_D$ +27°, which was separated through its ether-soluble hydrochloride, m. p. 260—261° (decomp.), $[\alpha]_D$ +37°, and in which configuration at $C_{(6)}$ is assigned provisionally by analogy with that established for 3 : 5-*cyclo*cholestan-6β-ol (Shoppee and Summers, *J.*, 1952, 3361; cf. Corey, Sneen, Danaher, Young, and Rutledge (*Chem. and Ind.*, 1954, 1294). The proportion of 3 : 5-*cyclo*-base formed by solvolysis appears to vary with the conditions, a lower temperature or a shorter reaction time leading to more 3 : 5-*cyclo*-base.


(IX)


(X)


(XI)


(XII)

The configuration of the bases (VI), (VII), (VIII), now established directly, confirms the assignment made by Haworth, McKenna, and Powell (*loc. cit.*), and supports the configurations assigned to the epimeric bases (X), (XI), (XII) by the workers, partly from the evidence of various Hofmann degradation experiments, and partly from the fact that the more thermodynamically stable equatorial epimerides tend to be produced by reduction of oximes of steroid ketones by use of sodium–ethanol.

Experimental

For general experimental directions see *J.*, 1954, 4224. $[\alpha]_D$ are in $CHCl_3$; Al_2O_3 used was Spence type H, 200 mesh, activity ~II, neutralised when necessary by Reichstein and Shoppee's procedure (*Discuss. Faraday Soc.*, 1949, **7**, 305).

Cholest-5-ene-3β-carboxyhydrazide.—Cholest-5-ene-3β-carboxylic acid (m. p. 222—223°; 1·22 g.) (Roberts, Shoppee, and Summers, *loc. cit.*) was refluxed with hydrazine hydrate (100%; 8 c.c.) in ethanol for 40 hr. Part of the solvent was removed by distillation and the hydrazide separated on cooling; recrystallisation from ethanol gave *cholest-5-ene-3β-carboxyhydrazide* as rectangular plates, m. p. 214—215°, $[\alpha]_D$ −24° (*c*, 1·39) [Found (after drying at 100°/0·01 mm. for 3 hr.): N, 5·8. $C_{28}H_{48}ON_2$ requires N, 6·5%].

Cholest-5-en-3β-yl iso*Cyanate.*—(*a*) The hydrazide (250 mg.) was dissolved in benzene (15 c.c.) and acetic acid (35 c.c.), the solution cooled to 0°, and 2N-hydrochloride acid (0·35 c.c.) added; a solution of sodium nitrite (95 mg.) in water (4 c.c.) was added dropwise and the mixture stirred at 0° for 1·5 hr. The mixture was diluted, and the benzene extract separated, washed with sodium hydrogen carbonate solution, and with water, and refluxed for 1 hr. to ensure complete conversion into the *iso*cyanate; this was obtained by evaporation as a crystalline solid, m. p. 80—96°, which could not be satisfactorily purified by recrystallisation.

(*b*) Cholest-5-ene-3β-carboxylic acid (1·22 g.), dissolved in benzene, was refluxed with thionyl chloride (1·5 c.c.) for 2 hr.; complete evaporation in a vacuum gave the crude chloride, m. p. 110—117°. The chloride, dissolved in *dry* acetone (50 c.c.) and dioxan (25 c.c.; freshly distilled over sodium), was treated dropwise with a solution of sodium azide (430 mg.) in water (3·5 c.c.) with stirring. After 10 min. the mixture was diluted, and the precipitate filtered off, washed with water, and dried in a vacuum-desiccator. The material was extracted with pentane, in which it was completely soluble (absence of original carboxylic acid), and the product obtained by evaporation dissolved in benzene and refluxed for 1 hr. to ensure conversion into the *iso*cyanate; this was obtained by evaporation as a crystalline solid, m. p. 80—90°, undepressed on admixture with the specimen obtained by procedure (*a*).

3β-*Aminocholest-5-ene.*—(i) The crude *iso*cyanate [625 mg. prepared by method (*b*)] was refluxed with 10% ethanolic potassium hydroxide (45 c.c.) for 1·5 hr.; the cooled solution was filtered from insoluble material (282 mg.), m. p. >250°, and evaporated completely. The residue was dissolved in ether, and the base isolated as the insoluble hydrochloride by treatment with dry hydrogen chloride. Basification with 2N-potassium hydroxide, ether-extraction, washing, drying, and evaporation furnished the amine as an oil, which crystallised overnight.

Part of the product by repeated recrystallisation from ether–pentane gave 3β-aminocholest-5-ene (VI), m. p. 89—94°, $[\alpha]_D$ −26° (*c*, 0·9), whilst part was purified by chromatography on a column of aluminium oxide (5 g.). Elution with ether–pentane (1 : 1) removed some non-basic material, whilst use of acetone–ether afforded a crystalline compound; recrystallisation from acetone gave a compound, m. p. 139—140°, $[\alpha]_D$ −37° (*c*, 0·87) [Found (after sublimation at 120°/0·01 mm.): C, 84·0; H, 12·4; N, 3·6%], which we regarded as a polymorph of the base (VI), but which has been shown by Haworth *et al.* (personal communication) to be the *iso*-propylidene derivative of the base (VI). The compound was converted by heating with acetic anhydride into the acetyl derivative, m. p. 238—239° (from ether), $[\alpha]_D$ −41° (*c*, 1·01), of the base (VI). The acetyl derivative (90 mg.) was shaken with Adams catalyst (18·5 mg.) in hydrogen; absorption (7·5 c.c.; calc. for 1 double bond and reduction of the catalyst, 8·1 c.c.) was complete in 3 hr. and the usual working up gave hexagonal plates of 3β-acetamidocholestane (68 mg.), m. p. 242—243°, $[\alpha]_D$ +11·5° (*c*, 1·06), after recrystallisation from ethanol. The m. p. was undepressed on admixture with the acetylated base obtained by reduction of cholestan-3-one oxime with sodium–ethanol.

(ii) The crude *iso*cyanate [237 mg.; prepared by method (*a*)] was hydrolysed and the crude base (114 mg.) isolated as under (i); the base was acetylated by brief heating with acetic anhydride (3 c.c.), and the product obtained by complete evaporation chromatographed on a column of aluminium oxide (5 g.). Elution with ether furnished 3β-acetamidocholest-5-ene (80 mg.), m. p. 238°, after recrystallisation from acetone–ether, undepressed on admixture with the specimen prepared by method (i).

3β-*Methylaminocholest-5-ene.*—(*a*) The crude *iso*cyanate (600 mg.), dissolved in ether, was treated with lithium aluminium anhydride (175 mg.), heated under reflux for 1 hr., cooled, and carefully diluted with water. The precipitate of aluminium hydroxide was filtered off, and the base isolated from dry ethereal solution as the hydrochloride, m. p. 222—226° (decomp.), $[\alpha]_D$ −27° (*c*, 1·21), after crystallisation from chloroform–acetone, this salt being basified with 2N-sodium carbonate. The product was chromatographed on aluminium oxide (5 g.); elution with ether–pentane (1 : 1) gave 3β-methylaminocholest-5-ene, m. p. 111—113°, $[\alpha]_D$ −37·5° (*c*, 0·8), after recrystallisation from acetone, undepressed on admixture with the specimen prepared by method (*b*) below.

(*b*) Cholesteryl toluene-*p*-sulphonate (5 g.) and methylamine (5 c.c. of 33% w/w solution in ethanol) were heated in a sealed tube at 100° for 8 hr.; the mixture was evaporated, and the product basified with ethanolic potassium hydroxide, diluted, and extracted with ether. The dry ethereal extract was treated with dry hydrogen chloride, and the precipitate collected and basified to give the amine (250 mg.) which after chromatographic purification (elution with ether–benzene, ether, acetone–ether, and acetone) furnished 3β-methylaminocholest-5-ene (140 mg.), m. p. 113°, as needles, after recrystallisation from acetone; methylation of a specimen (32 mg.) with formaldehyde (0·5 c.c.; 40%), formic acid (0·5 c.c.; 90%), and water (1 c.c.) at 100° for 4 hr. gave 3β-dimethylaminocholest-5-ene, m. p. 151—152°, $[\alpha]_D$ −32° (*c*, 0·76), after recrystallisation from acetone. The ethereal filtrate was evaporated and the residue extracted with pentane to furnish a solid, which by recrystallisation from acetone gave material (770 mg.), m. p. 80—88°, $[\alpha]_D$ −28° (*c*, 1·09), which is possibly 3α-methylaminocholest-5-ene (cf. Haworth, McKenna, and Powell, *loc. cit.*); the residue of ether-soluble hydrochloride gave a crude base (1·12 g.), which was purified by chromatography on aluminium oxide (30 g.) prepared in pentane. Elution with 50 c.c. portions of pentane, benzene–pentane (1 : 1), benzene, ether–benzene (1 : 1), and ether gave fractions of m. p. 52—54° (45 mg.), 52—54° (413 mg.), 51—53° (66 mg.), 47—51° (166 mg.), and 48—52° (90 mg.). Recrystallisation from acetone gave 6β-*methylamino*-3 : 5-cyclo*cholestane*, m. p. 52—54°, $[\alpha]_D$ +27°, +28° (*c*, 0·98, 1·18) [Found (after distillation at 150°/0·01 mm.): C, 83·6; H, 12·2. $C_{28}H_{49}N$ requires C, 84·1; H, 12·4%]; the hydrochloride, obtained by precipitation with hydrogen chloride from pentane solution, crystallised from chloroform–acetone in needles, m. p. 260—261° (decomp.), $[\alpha]_D$ +37° (*c*, 0·78).

One of us (R. H. P.) acknowledges a grant from the Swansea Local Education Authority; we thank Glaxo Laboratories Ltd. for a gift of cholesterol.

UNIVERSITY OF WALES, UNIVERSITY COLLEGE, SWANSEA [*Received, September 18th,* 1954.]