103. *Aminosteroids. Part I. α- and β-Forms of 7-Aminocholesterol.*

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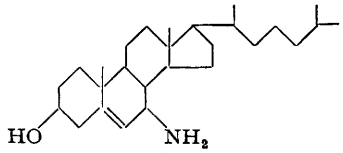
The α- and *β-isomers* of 7-aminocholesterol have been isolated by fractional crystallisation of the mixture obtained by reduction of the oxime of 7-ketocholesteryl acetate. The isomeric acetyl derivatives have also been separated, but all attempts to hydrolyse them to the free bases have failed. *α-7-Aminocholestanol* has been obtained by catalytic reduction of α-7-aminocholesterol. Both the α- and β-7-aminocholesterol isomers show high antibacterial activity *in vitro* against Gram-positive organisms, but no appreciable difference has been observed between the activities of the two spatial isomers.

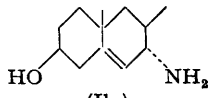
The preparation of various aminosterols was undertaken with a view to examining their activity as antibacterial agents. 7-Aminocholesterol (I) was chosen as the first member of the series to be examined, since it had already been made, in the form of a mixture of α- and β-isomers, by reduction of the oxime of 7-ketocholesteryl acetate with sodium in alcohol (Eckhardt, *Ber.*, 1938, **71**, 461). Eckhardt described the separation of the α- and β-*N*-acetyl derivatives by fractional crystallisation but did not succeed in separating the free bases. The above work has now been repeated; the two *N*-acetyl derivatives obtained by fractional crystallisation have been found to have melting points somewhat higher than those described by Eckhardt. It was not found possible to hydrolyse the *N*-acetyl compounds to the free bases, for alkaline conditions left the *N*-acetyl group untouched and acid treatment either effected no change or removed the amino group com-

pletely. However, the free aminosterols were eventually separated by direct fractional crystallisation from methyl alcohol–acetone. The mixture of isomers was first partially separated by elution from a column of alumina, by which means a seed crystal of the pure β-form was obtained. Since the latter is the less soluble of the two forms, it could be almost completely removed first by careful fractional crystallisation and the α-form could then be isolated from the mother liquors.

The nomenclature adopted here for the 7-amino-isomers is that corresponding to the 7-*N*-acetyl derivatives to which they give rise respectively, following Eckhardt's arbitrary designation for the latter (*loc. cit.*).



(Ia.)



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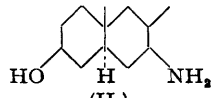

(II.)

α-7-*Aminocholesterol* (Ia) crystallises from methyl alcohol–acetone in diamond-shaped prisms, m. p. 183—185°, $[\alpha]_D^{20°}$ −327° in chloroform. The β-*form* (Ib) crystallises as cubes or rectangular plates, m. p. 198—199°, $[\alpha]_D^{20°}$ +181° in chloroform. The conversion of both isomers into the corresponding *N*-acetyl derivatives having the melting points and rotations already found for the separated isomers confirms the completeness of the separation. The optical rotation of α-7-aminocholesterol appears to be one of the highest values recorded for any steroid.

The free aminosterols are sufficiently strong bases to take up carbon dioxide from the air; all crystallisations and filtrations were therefore carried out in an atmosphere of nitrogen. The bases can be precipitated quantitatively as their hydrochlorides by addition of dry ethereal hydrogen chloride to their solutions in ether. The salts are insoluble in water but are not precipitated from alcoholic solution by addition of water, soap-like, colloidal solutions being obtained (see also Eckhardt, *loc. cit.*).

The α- and β-7-aminocholesterol isomers have been catalytically reduced, in glacial acetic acid solution in the presence of platinum black, to the corresponding α- and β-7-aminocholestanol isomers. Of these, only the α-form (II) (analysed as the *hydrochloride*) has been obtained crystalline.

Biological results. Substances for testing were in the form of their hydrochlorides, made up in a 10% alcoholic solution. Dilutions of from 1 : 10^3 to 1 : (5 × 10^5) were tested in synthetic medium, glucose broth, and serum broth, against *Streptococcus hæmolyticus*, *Staphylococcus aureus*, *B. coli*, and *Ps. pyocyanea*. Results are shown in the Table.

Limiting dilution giving complete inhibition of growth.

Organism.	*Staph. aur.*	*Strep. hæm.*	*B. coli.*	*Ps. pyoc.*
Synthetic medium.				
α-7-Aminocholesterol	1 : (5 × 10^5)	1 : 10^5	1 : 10^3	nil.
β-7-Aminocholesterol	1 : (5 × 10^5)	1 : (5 × 10^5)	nil.	nil.
Mixed α- and β-isomers	1 : 10^5	1 : 10^5	nil.	nil.
α-7-Aminocholestanol	1 : 10^5	1 : (5 × 10^5)	1 : 10^3	nil.
Glucose broth.				
α-7-Aminocholesterol	1 : 10^5	1 : 10^5	nil.	nil.
β-7-Aminocholesterol	1 : 10^5	1 : (5 × 10^5)	nil.	nil.
Mixed α- and β-isomers	1 : 10^5	1 : 10^5	nil.	nil.
α-7-Aminocholestanol	1 : (5 × 10^4)	1 : (5 × 10^4)	nil.	nil.
Serum broth.				
α-7-Aminocholesterol	1 : 10^4	1 : 10^5	nil.	nil.
β-7-Aminocholesterol	1 : 10^4	1 : 10^5	nil.	nil.
Mixed α- and β-isomers	1 : 10^4	1 : 10^4	nil.	nil.
α-7-Aminocholestanol	1 : 10^4	1 : 10^4	nil.	nil.

It will be seen that all the substances showed appreciable antibacterial activity against the Gram-positive organisms, although the activity was somewhat reduced in serum broth. However, there does not seem to be any specific activity connected with the spatial configuration of the basic group in position 7. Some difference might have been expected if the antibacterial potency is in any way connected with the surface activity.

Experimental.

α- *and* β-7-*Aminocholesterol.*—The mixture of isomers was isolated as the hydrochloride exactly as described by Eckhardt (*loc. cit.*), and the free bases obtained by extraction from alkaline solution with freshly redistilled ether. Crystallisation from methyl alcohol–acetone gave a *ca.* 40% yield of the mixed free bases, m. p. 170—185°.

α- *and* β-7-N-*Acetamidocholesterol.*—The mixed 7-amino-bases (2·0 g.) were dissolved in absolute ether (400 c.c.) and a solution of acetic anhydride (6 c.c.) in ether (40 c.c.) added. A crystalline mass separated after a few minutes. It was left at room temperature overnight, then filtered off and washed with absolute ether. The α-form was isolated first by virtue of its low solubility in absolute alcohol. By careful fractionation of the ethyl alcoholic mother liquors obtained after removing most of the relatively insoluble α-form, a fairly complete separation of the two forms was achieved, *viz.* 705 mg. of the α-form and 980 mg. of the β-form, from 2 g. of the original bases. The α-*isomer* formed glistening leaflets after recrystallisation from chloroform–methyl alcohol, m. p. 291—293° (decomp.) unchanged by two further recrystallisations, $[\alpha]_D^{19°}$ −183° (*c*, 0·2 in 1 : 1 chloroform–methyl alcohol) (Found : N, 3·13. Calc. for $C_{29}H_{49}O_2N$: N,

3·16%). The *β-form* was obtained as large regular rectangular prisms after recrystallisation from absolute alcohol, m. p. 277—279° (decomp.) unchanged by two further recrystallisations, $[\alpha]_D^{15°}$ +81° (*c*, 1 in 1 : 1 chloroform–methyl alcohol) (Found : N, 3·27%.) A mixture of the two isomers had m. p. 261—264°.

Attempted Hydrolysis of 7-N-*Acetamidocholesterol.*—(*a*) Treatment of the *N*-acetyl derivative (50 mg.) with 4% methyl alcoholic sodium hydroxide solution, refluxing for 2 hours, yielded the starting material unchanged (35 mg.), m. p. 290—291°.

(*b*) Treatment with 30% methyl alcoholic sodium methoxide for 16 hours in boiling solution gave a small amount of unchanged material. The residue could not be crystallised.

(*c*) Treatment of the β-isomer (50 mg.) with methyl alcohol–concentrated hydrochloric acid (1 : 1 mixture), for 1½ hours in boiling solution, gave the unchanged β-isomer (20 mg.) after recrystallisation, m. p. 277—279°.

(*d*) Treatment as for (*c*), but boiling for 16 hours. Extraction of the acid solution with ether gave non-basic material (35 mg.) which contained no nitrogen. Ammonium chloride had sublimed on the condenser, hence this treatment apparently removed the amino group completely. The acid-soluble fraction, on being made alkaline and extracted with ether, yielded only 4 mg. of basic material.

(*e*) Treatment with 4% methyl alcoholic hydrogen chloride in a sealed tube at 80° overnight, followed by 6 hours' heating at 130°, gave unchanged starting material.

(*f*) Treatment as in (*e*), leaving at 15° for 3 days, also effected no change.

(*g*) Treatment with 10% methyl alcoholic hydrochloric acid again yielded unchanged material.

In view of these results attempts at hydrolysis were abandoned.

Attempted Separation of the Free Bases on a Column of Alumina.—The mixed free base (250 mg.) was adsorbed on a small tower of alumina in a small volume of benzene. The tower was eluted successively with light petroleum (b. p. 40—60°) (*ca.* 200 c.c.), and then with benzene, *ca.* 50 c.c. of eluate being collected each time. After *ca.* 200 c.c. of benzene had passed through, the free base was obtained from the eluate in three successive fractions. The first of these, on crystallisation from methyl alcohol–acetone, yielded the β-isomer as cubes, m. p. 193—195°; the other two fractions gave mixtures of α- and β-forms.

Fractional Crystallisation.—The mixed 7-amino-isomers (7 g.) were dissolved in methyl alcohol (90 c.c.), and acetone (50 c.c.) was added. After seeding with a crystal of the β-form and leaving for 30 minutes, a crop of cubes (β-form) was obtained (2·08 g.), m. p. 189—192° rising to 198—199° after two more recrystallisations and unchanged by further crystallisation. Concentration of the filtrate to half the bulk yielded a mixture of cubes and diamonds (2·5 g.). The mother liquors on standing, after seeding with a crystal of the α-form, gave the pure α-isomer as diamond-shaped prisms (2·46 g.), m. p. 183—185° unchanged by further recrystallisation from methyl alcohol–acetone. The α-form appears to exist in two crystalline modifications; when the diamond-shaped prisms (m. p. 183°) were recrystallised from methyl alcohol alone, hair-like needles came down, m. p. 140—142°, and were much more soluble in acetone than the prisms. Recrystallisation of these needles from acetone again gave the diamonds, m. p. 183—185°.

α-7-*Aminocholesterol* had m. p. 183—185°, $[\alpha]_D^{20°}$ −327° (*c*, 0·74 in chloroform) (Found : N, 3·42. $C_{27}H_{47}ON$ requires N, 3·49%). Second form : needles, m. p. 140—142°, $[\alpha]_D^{20°}$ −80° (*c*, 0·94 in 1 : 1 chloroform–methyl alcohol).

The α-amino-isomer (37 mg.), dissolved in absolute ether (10 c.c.), was treated with acetic anhydride (0·12 c.c.) and left overnight at 10°. This yielded α-7-*N*-acetamidocholesterol (33 mg.), m. p. 291—293°, giving no depression with that isolated from the original mixture of *N*-acetyl derivatives, $[\alpha]_D^{20°}$ −194° (*c*, 0·5 in 1 : 1 chloroform–methyl alcohol).

β-7-*Aminocholesterol* had m. p. 198—199°, $[\alpha]_D^{20°}$ +181° (*c*, 0·72 in chloroform) (Found : N, 3·44%).

The β-7-amino-isomer (40·0 mg.) was acetylated as described for the α-form. β-*N*-Acetamidocholesterol was obtained (30·0 mg.), m. p. 277—279° (decomp.), $[\alpha]_D^{18°}$ +85° (*c*, 0·56 in 1 : 1 chloroform–methyl alcohol).

From the final mother liquor of the 7-aminocholesterol isomers a crystalline, non-nitrogenous material was isolated as clusters of fine needles, m. p. 175—176° after two recrystallisations from acetone. It gave a considerable depression of m. p. both with the 7-amino-isomers and with the original oxime. The analysis was correct for a 7-hydroxycholesterol (Found : C, 80·48; H, 11·49. Calc. for $C_{27}H_{46}O_2$: C, 80·59; H, 11·53%). This could presumably only arise by reduction and simultaneous hydrolysis of 7-ketocholesteryl acetate which might have been present in a small quantity in the oxime used.

α-7-*Aminocholestanol.*—α-7-Aminocholesterol (830 mg.), dissolved in glacial acetic acid (20 c.c.), was hydrogenated in the presence of a platinum black catalyst (0·3 g.) at atmospheric pressure. The theoretical amount of hydrogen (2 atoms = 44 c.c.) was taken up in four minutes. The filtrate was concentrated under reduced pressure to small bulk, neutralised with sodium carbonate solution, made alkaline with sodium hydroxide, and extracted three times with ether. The ethereal extracts, after washing with dilute alkali and drying (Na_2SO_4), were evaporated to dryness (weight 820 mg.). The residue was readily soluble in ethyl and methyl alcohols and in light petroleum. It was crystallised by dissolving in boiling 80% aqueous ethyl alcohol; on cooling flocks of needles appeared, m. p. 84—86° to an opaque liquid. Yield of pure material, 645 mg. Further recrystallisation raised the m. p. to 87—89° (sintered at 84°), $[\alpha]_D^{16°}$ −143° (*c*, 1·1 in absolute alcohol). Addition of ethereal hydrogen chloride to a solution of this base (400 mg.) in absolute ether precipitated the *hydrochloride* as a dry white powder (420 mg.) (Found : N, 3·22. $C_{27}H_{49}ON,HCl$ requires N, 3·18%).

β-7-*Aminocholestanol.*—Reduction of the β-isomer proceeded exactly as described for the α-form. The product, however, has not yet been obtained crystalline.

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