105. *Aminosteroids. Part III. Some Mono- and Di-aminosteroids.*

By Jean Barnett, Brenda E. Ryman, and F. Smith.

Various mono- and di-aminosteroids have been prepared by reduction of the oximes of the corresponding mono- and di-ketosteroids. Examination of their properties as antibacterial agents *in vitro* against Gram-positive organisms showed a marked and similar activity in all compounds studied. Only the diaminosteroids had any appreciable bacteriostatic activity against Gram-negative organisms.

In continuation of the study of aminosteroids (Part I, Barnett, Ryman, and Smith, this vol., p. 524) various mono- and di-aminosteroids have been made by reduction of the corresponding mono- and di-oximes. Table I shows the ketones which were used as starting products, together with their oximes, with reference to their method of preparation when they were already known. No difficulty was experienced in the synthesis of any

of the oximes except that from 6 : 7-diketocholestanyl acetate; presumably steric hindrance retarded its formation, for complete oximation was effected only after refluxing the components for 48 hours.

TABLE I.

Ketone.	M. p.	Reference.	Oxime.	Reference.
6-Ketocholestanyl acetate	122—124°	Heilbron *et al.*, *J.*, 1938, 104.	201—202°	—
Cholestenone	79—80	*Helv. Chim. Acta*, 1934, **17**, 1413.	152	*Ber.*, 1904, **37**, 3101.
7-Ketocholesteryl acetate	153—154	Windaus, Lettré, and Schenk, *Annalen*, 1935, **520**, 98.	184—185	Eckhardt, *Ber.*, 1938, **71**, 467.
3 : 7-Diketocholestene	184—185	Barnett, Ryman, and Smith, Part II, this vol., p. 526.	229—230	Barnett, Ryman, and Smith, *loc. cit.*
3 : 6-Diketocholestane	169—170	Windaus, *Ber.*, 1903, **36**, 3755.	205—210	Windaus, *loc. cit.*
6 : 7-Diketocholestanol	152—153	—	245—247	—

Biological Results.—Monoaminosteroids were prepared as their hydrochlorides for testing; diaminosteroids were in the form of their dilactates, since their dihydrochlorides were too insoluble to be of use. Dilutions of from 1 : 10^3 to 1 : (5 × 10^5) were tested *in vitro* in synthetic media, glucose broth, and (a few) in serum broth, against *Streptococcus hæmolyticus*, *Staphylococcus aureus*, *B. coli*, and *Ps. pyocyanea*. Results are shown in Table II. Results with 7-aminocholesterol are included for the comparison.

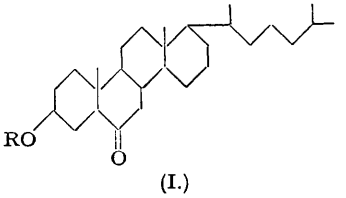
TABLE II.

Limiting dilution giving complete inhibition of growth.

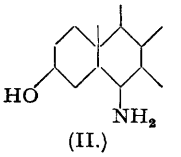
Synthetic medium.	*Strep. hæm.*	*Staph. aur.*	*B. coli.*	*Ps. pyoc.*
3-Aminocholestene [1]	1 : 10^4	1 : 10^4	nil.	nil.
3-Aminocholestane [2]	1 : 10^5	1 : 5 × 10^4	1 : 10^3	nil.
6-Aminocholestanol	1 : 10^5	<1 : 10^5	<1 : 10^3	<1 : 10^3
7-Aminocholesterol (β)[3]	1 : (5 × 10^5)	1 : (5 × 10^5)	nil.	nil.
7-Dimethylaminocholesterol [4]		Not tested.		
3 : 7-Diaminocholestene	1 : 10^5	1 : (5 × 10^5)	1 : 10^4	1 : 10^4
3 : 6-Diaminocholestane	1 : 10^5	1 : (5 × 10^5)	1 : 10^4	nil.
6 : 7-Diaminocholestanol	1 : 10^5	1 : 10^5	1 : (5 × 10^3)	1 : 10^3
Glucose broth.				
3-Aminocholestene	1 : (5 × 10^3)	1 : (5 × 10^3)	nil.	nil.
3-Aminocholestane	1 : 10^4	1 : (5 × 10^3)		
6-Aminocholestanol	<1 : 10^5	<1 : 10^5	1 : 10^4	1 : (5 × 10^3)
7-Aminocholesterol	1 : (5 × 10^5)	1 : 10^5	nil.	nil.
7-Dimethylaminocholesterol		Not tested.		
3 : 7-Diaminocholestene	1 : 10^5	1 : (5 × 10^4)	1 : 10^4	1 : (5 × 10^3)
3 : 6-Diaminocholestane	1 : 10^5	1 : (5 × 10^5)	1 : 10^4	1 : (5 × 10^3)
6 : 7-Diaminocholestanol	1 : 10^5	1 : 10^5	1 : 10^3	1 : 10^3
Serum broth.				
3-Aminocholestene	1 : (5 × 10^3)	1 : (5 × 10^3)	nil.	nil.
3 : 7-Diaminocholestene	1 : (5 × 10^4)	1 : (5 × 10^3)	1 : (5 × 10^3)	1 : 10^3

[1] Windaus and Adamla, *Ber.*, 1911, **44**, 3051. [2] Diels and Stamm, *Ber.*, 1912, **45**, 2232. [3] Part I, *loc. cit.* Eckhardt, *Ber.*, 1938, **71**, 467.

It will be seen from Table II that all the aminosteroids so far tested are highly active *in vitro* against *Streptococci* and *Staphylococci*; on the whole the diaminosteroids appear to possess a consistently higher antibacterial potency than the monoaminosteroids. Moreover, the group of diamino compounds shows considerable bacteriostatic activity against the Gram-negative organisms tested. *In vivo* tests on some of these bases are in progress. It is hoped to continue the investigation to cover triaminosteroids.



(I.)



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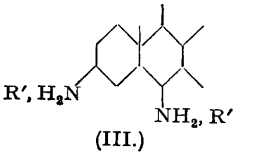

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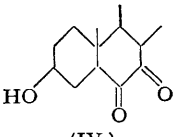

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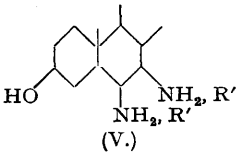

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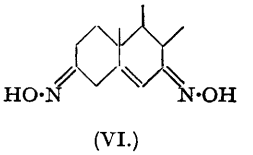

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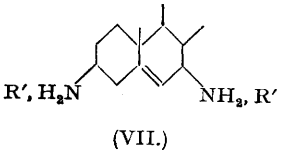

(VII.)

R = Ac. R' = Lactic acid.

Experimental.

6-Ketocholestanyl Acetate (I, R = Ac).—This was prepared by the method of Heilbron *et al.* (*J.*, 1938, 104). The purity of the nitration product was found to depend very much on the strength of the fuming nitric acid used, and on the efficiency of the stirring.

Oxime of 6-Ketocholestanyl Acetate.—6-Ketocholestanyl acetate (200 mg.) was treated with hydroxylamine hydrochloride and sodium acetate, refluxing in alcoholic solution for 8 hours. On cooling, the oxime settled out as glistening plates. Recrystallisation from aqueous alcohol gave the *oxime* (150 mg.), m. p. 201—202° (Found: C, 75·95; H, 10·72; N, 3·31. $C_{29}H_{49}O_2N$ requires C, 75·82; H, 10·68; N, 3·05%).

6-Aminocholestanol hydrochloride (II) was obtained by reduction of the oxime as described previously (Found: N, 3·18. $C_{27}H_{49}ON,HCl$ requires N, 3·20%). It was soluble in alcohol and insoluble in water.

3 : 6-Diketocholestane.—This was prepared by oxidation of 6-ketocholestanol as described by Windaus (*loc. cit.*). The dioxime, prepared as already described, had m. p. 205—210° (decomp.).

3 : 6-Diaminocholestane dilactate (III) was prepared by reduction of the dioxime in the usual manner (Found: N, 4·31. $C_{27}H_{50}N_2,2C_3H_6O_3$ requires N, 4·79%). It was readily soluble in alcohol and water.

6 : 7-Diketocholestanol (IV).—The acetate was prepared from 7-bromo-6-ketocholestanyl acetate by treatment with silver nitrate and pyridine (Heilbron *et al.*, *J.*, 1937, 803); it had m. p. 156—157°.

6 : 7-Diketocholestanyl acetate (500 mg.) was dissolved in warm 2% methyl alcoholic sodium hydroxide solution (10 c.c.) and refluxed for 15 minutes. Water was added, then dilute sulphuric acid until the solution was acid. After extracting the product three times with ether and washing well with water, evaporation of the ethereal extracts yielded a crystalline product which was recrystallised from methyl alcohol by addition of water giving *6 : 7-diketocholestanol* as needles (400 mg.), m. p. 149—151°. Repeated recrystallisation raised the m. p. to 152—153°. The compound gave a deep green colour with alcoholic ferric chloride (Found: C, 75·46; H, 10·60. $C_{27}H_{44}O_3$ requires C, 75·0; H, 10·8%).

Dioxime of 6 : 7-Diketocholestanyl Acetate.—Preparation of this oxime gave some difficulty; treatment for three hours or eight hours with hydroxylamine gave unchanged diketone; the following method was finally used: 6 : 7-diketocholestanyl acetate (200 mg.) was refluxed for 48 hours in alcoholic solution with four times the theoretical amount of hydroxylamine. The end of the reaction could be ascertained when no crystalline material separated on cooling, since the oxime was extremely soluble in alcohol. Addition of water to the alcoholic solution caused the separation of the *dioxime* as fine white needles, m. p. 242—244° (110 mg.). A further crop was obtained on concentration of the filtrate (80 mg.; m. p. 240—241°). Recrystallisation from aqueous methyl alcohol gave fine feathery needles, m. p. 245—247° (Found: C, 71·97, 71·90; H, 10·01, 9·96; N, 5·59. $C_{29}H_{48}O_4N_2$ requires C, 71·32; H, 9·91; N, 5·73%).

6 : 7-Diaminocholestanol Dilactate (V).—This was prepared in the usual way by reduction of the dioxime (880 mg.), yielding the *dilactate* as a white deliquescent powder (603 mg.) (Found: N, 4·78. $C_{27}H_{50}ON_2,2C_3H_6O_3$ requires N, 4·7%). It was soluble in water and alcohol but insoluble in ether.

3 : 7-Diaminocholestene (VII).—The steroid base was obtained by reduction of 3 : 7-diketocholestene dioxime (VI) (Part II, *loc. cit.*) with sodium in ethyl alcoholic solution. 4 G. of dioxime yielded 3·9 g. of the diamino-dihydrochloride. As the dihydrochloride was insoluble in alcohol as well as in water, and would therefore be useless for testing, it was transformed *via* the free base (which was not isolated since it would be a mixture of four possible isomers) into the dilactate by addition of two mols. of lactic acid in ethereal solution to the solution of the base in dry ether. The dilactate was precipitated as a yellowish powder, which was readily soluble in cold alcohol and soluble in water on slight warming, but which could not be recrystallised.

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