The Synthesis and Antibacterial Activity of Some Basic Derivatives of the Bile Acids.

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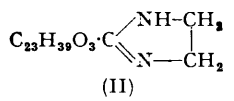
Two series of basic derivatives of the bile acids have been synthesised, the first by the reaction of the methyl esters of certain bile acid derivatives with ethylenediamine hydrate and the second by esterification of 3-amino-7α : 12α-dihydroxycholanoic acid hydrochloride with a number of alcohols. The bacteriostatic activity of the compounds against *Staphylococcus aureus* and *Aerobacter aerogenes*, and their ability to depress the surface tension of the culture media of these organisms, showed no relation between the two properties for the ethylenediamine derivatives, but revealed a striking parallelism for the second set of esters.

STACEY and WEBB (*Proc. Roy. Soc.*, 1947, *B*, **134**, 523) showed that the antibacterial activity of certain anionic bile acid derivatives depended on their surface activity: the depression of the surface tension of the culture medium by these compounds at their limiting bacteriostatic concentration for *Staphylococcus aureus* was in every case approximately 4·5 dynes/cm. No such relation existed, however, for a series of cationic bile acid derivatives (Stacey and Webb, *ibid.*, p. 538). The anionic derivatives used in these investigations had closely related structures in that they all contained a carboxyl group in the bile acid side chain. The cationic derivatives, however, varied in that some contained amino-, amidino-, and guanidino-groups in the bile acid side chain, while others contained amino-groups in the nucleus. It appeared desirable, therefore, to study more closely related compounds. Methods were sought for producing such compounds without resorting to the tedious production of 23- and 24-amino-derivatives (James, Smith, Stacey, and Webb, *J.*, 1946, 665; Hilton and Webb, *J.*, 1951, 2767).

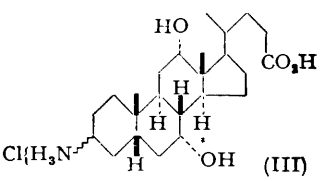
First, methyl esters of certain bile acid derivatives were treated with boiling ethylenediamine hydrate, giving the required basic derivatives in one step. Methyl cholate thus gave *N*-3α : 7α : 12α-trihydroxycholanoylethylenediamine. From the analytical data and the fact that the compound reacted as a primary and not as a secondary amine (Feigl and Anger, *Mikrochim. Acta*, 1937, **1**, 138) it was considered to have the open-chain structure (I) and not the ring form (II) which would result by loss of water (cf. Hill and Aspinall, *J. Amer. Chem. Soc.*, 1939, **61**, 822; Aspinall, *ibid.*, p. 3195). Methyl deoxycholate, lithocholate, apocholate, 3α : 12α-dihydroxychol-14-enoate, and 3-amino-7α : 12α-dihydroxycholanoate hydrochloride gave similar products; with the unsaturated derivatives there was the possibility of migration of the double bond, but its position in the final product was not ascertained.

$C_{23}H_{39}O_3{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}NH_2$

(I)



(II)



(III)

A second method was simply to esterify 3-amino-7α : 12α-dihydroxycholanoic acid hydrochloride (III) (Jones, Webb, and Smith, *J.*, 1949, 2164) with a number of straight-chain and branched-chain alcohols. (Syntheses of the methyl and *iso*pentyl esters have already been reported; Jones, Webb, and Smith, *loc. cit.*)

These compounds were tested for antibacterial activity against *Staph. aureus* and *Aerobacter aerogenes* and for their ability to depress the surface tension of the culture media of these organisms.

It was apparent (Tables 2 and 3) that there was no close relation between the bacteriostatic activities and surface activities of the ethylenediamine derivatives. Thus, at the

limiting dilution which was bacteriostatic for *Staph. aureus* the depression of surface tension varied from 6·0 dynes/cm. for *N*-3α : 7α : 12α-trihydroxycholanoylethylenediamine to 2·5 dynes/cm. for *N*-3α-hydroxycholanoylethylenediamine. Moreover, the compounds with the highest surface activity did not show the highest bacteriostatic activity. There did appear to be, however, a relation between the bacteriostatic activity and surface activity of the esters of 3-amino-7α : 12α-dihydroxycholanoic acid hydrochloride. Thus, as the carbon chain was increased, the antibacterial activities of the compounds increased in the same order as their surface activities, both rising to a maximum at the *n*-hexyl and *iso*pentyl esters and, for eight of the twelve esters studied, the values for the depression of the surface tension at the limiting dilution which was bacteriostatic for *Staph. aureus* were between 13·2 and 11·2 dynes/cm. (mean = 12·2; $\sigma \pm 0{\cdot}9$). It may be noted that the limiting dilutions varied from 1 : 256,000 for the *n*-hexyl ester (depression 13·2 dynes/cm.) to 1 : 8000 for the *n*-propyl ester (depression 13·2 dynes/cm.).

Similar results were obtained by Birchenough (*J.*, 1951, 1263) for a series of 2-alkylpyridinium salts in which maximum antibacterial action and maximum depression of surface tension occurred when the alkyl group was *n*-pentadecyl.

In considering these and other results Burton (*Sci. Prog.*, 1951, **39**, 253) pointed out that there was some correlation between antibacterial activity and surface activity in a homologous series of quaternary ammonium compounds, but that there was none between compounds of different series. He concluded that the antibacterial action of quaternary ammonium compounds depended partly on their specific structure and partly on surface activity, the latter effect predominating in a homologous series. The results obtained here with the esters of 3-amino-7α : 12α-dihydroxycholanoic acid may be explained on the same basis, the effect of surface activity predominating with the long-chain esters, but that due to specific structure predominating with the methyl and ethyl esters, both of which showed comparatively low surface activity at their limiting bacteriostatic concentrations (depression, 8·0 and 7·8 dynes/cm. respectively).

The *iso*hexyl and 2-ethylhexyl esters showed lower bacteriostatic activities than would be expected from their surface activities. This may have been due to the fact that at the limiting bacteriostatic concentrations these compounds formed a thick emulsion with the constituents of the culture medium, which may have interfered with determination of the surface tensions. A similar effect occurred at the limiting dilutions which were bacteriostatic for *Aerobacter aerogenes*, with many of the less active compounds, so no definite conclusions could be reached in this case. It may be noted, however, that for the more active derivatives, again with the exception of the ethyl ester, the depression of surface tension at the limiting bacteriostatic dilution varied only from 13·2 to 14·3 dynes/cm.

It appears from the high antibacterial activity of 23-amino-3α-hydroxynorcholane hydrochloride and many other basic bile acid derivatives (Stacey and Webb, *Proc. Roy. Soc.*, 1947, *B*, **134**, 538; Hilton and Webb, *J.*, 1951, 2767), compared with their low surface activity, that, in general, the bacteriostatic activity of the basic bile acid derivatives is not due to surface activity but to a " specific-structure effect " as postulated by Burton (*loc. cit.*). In the series of esters studied, however, a compound of comparatively low activity (*e.g.*, a methyl ester) was transformed into a compound of high activity (*e.g.*, a *n*-hexyl ester) by increasing the carbon chain, thus superimposing an effect which was due to surface activity. Some caution should be exercised, however, in relating antibacterial power directly to surface activity even in a homologous series, for although in such a series surface activities may parallel the bacteriostatic activities, there remains the possibility that both properties may be dependent upon a third and more fundamental variable.

Experimental

N-3α : 7α : 12α-*Trihydroxycholanoylethylenediamine Hydrochloride*.—A solution of methyl cholate (0·5 g.) in ethylenediamine hydrate (10 ml.) was boiled under reflux for 3 hr. The cooled solution was poured into water (200 ml.) and made strongly alkaline with 5N-sodium hydroxide, and the precipitated base filtered off after 18 hr. and dried *in vacuo* (P_2O_5–NaOH). Addition of 5N-hydrochloric acid to a suspension of the base in acetone, followed by the addition

of excess of acetone, precipitated N-3α : 7α : 12α-*trihydroxycholanoylethylenediamine hydrochloride* (0·1 g.), which crystallised from aqueous acetone as needles, m. p. 275° (sintering at 250°), $[\alpha]_D^{18}$ +37·6° (*c*, 0·94 in H_2O) (Found, after drying at 190°: C, 64·1; H, 9·6; N, 5·8. $C_{26}H_{46}O_4N_2$,HCl requires C, 64·1; H, 9·7; N, 5·8%).

N-3α : 12α-*Dihydroxycholanoylethylenediamine Hydrochloride.*—Methyl deoxycholate (1 g.) and ethylenediamine hydrate (10 ml.) were boiled under reflux for 3 hr. The solution was evaporated under reduced pressure and the crystalline residue dried *in vacuo* (H_2SO_4) and dissolved in chloroform (30 ml.). Dry hydrogen chloride was bubbled through this solution at 0° until no further precipitation was apparent. The supernatant solution was removed and the precipitate triturated with acetone until solid. Crystallisation from aqueous acetone gave N-3α : 7α-*dihydroxycholanoylethylenediamine hydrochloride* (300 mg.), needles, m. p. 177—178°, $[\alpha]_D^{20}$ +34·6° (*c*, 1·04 in H_2O) (Found, after drying at 160°: C, 65·9; H, 9·6; N, 6·3. $C_{26}H_{46}O_3N_2$,HCl requires C, 66·3; H, 10·0; N, 5·95%).

N-3α-*Hydroxycholanoylethylenediamine Hydrochloride.*—Methyl lithocholate (0·2 g.) in ethylenediamine hydrate was boiled under reflux for 3 hr. The gelatinous material obtained upon cooling was triturated with water until a granular solid was produced which was then suspended in water (100 ml.) and made alkaline to litmus with 5N-sodium hydroxide. The solid material was filtered off, washed with water, dried, and dissolved in acetone–ethanol (2 : 1) containing a few drops of 5N-hydrochloric acid. Addition of excess of acetone to the filtered solution precipitated a gelatinous solid which was dissolved in ethanol, and the solution evaporated under reduced pressure. Trituration of the resulting syrup with acetone–ethanol (4 : 1) gave crystals which, recrystallised from aqueous acetone, gave N-3α-*hydroxycholanoylethylenediamine hydrochloride* (75 mg.) as needles, m. p. 193—195° (sintering at 175°), $[\alpha]_D^{19}$ +16° (*c*, 1·0 in EtOH) (Found, after drying at 170°: N, 6·3. $C_{26}H_{46}O_2N_2$,HCl requires N, 6·2%).

Methyl *apo*cholate (1 g.) (Boedecker, *Ber.*, 1920, **53**, 1852; 1921, **54**, 2489; 1922, **55**, 2302) and methyl 3α : 12α-dihydroxychol-14(15)-enoate (100 mg.) (Boedecker, *loc. cit.*, 1921) were treated with ethylenediamine hydrate as described above and the products isolated essentially by the method used for the methyl deoxycholate product. N-3α : 12α-*Dihydroxychol*-8(14)-*enoyl*- (175 mg.), m. p. 145° (decomp.), $[\alpha]_D^{20}$ +26° (*c*, 1·0 in H_2O) (Found, after drying at 120°: C, 66·4; H, 9·7; N, 5·5. $C_{16}H_{44}O_3N_2$,HCl requires C, 66·6; H, 9·6; N, 6·0%), and N-3α : 12α-*dihydroxychol*-14(15)-*enoyl-ethylenediamine hydrochloride* (60 mg.), m. p. 148° (decomp.), $[\alpha]_D^{20}$ +37·4° (*c*, 1·01 in H_2O) (Found, after drying at 120°: C, 66·2; H, 9·6; N, 6·0%), were isolated (absence of migration of the double bonds being assumed).

N-3-*Amino*-7α : 12α-*dihydroxycholanoylethylenediamine Dihydrochloride.*—A solution of methyl 3-amino-7α : 12α-dihydroxycholanoate hydrochloride (400 mg.) in ethylenediamine hydrate (3 ml.) was boiled under reflux for 3 hr. The cooled solution was made strongly alkaline by 5N-sodium hydroxide, the product separating as an oil. The oil was centrifuged, the supernatant liquid removed, and the sediment dried *in vacuo* (P_2O_5–H_2SO_4). The dried material was then dissolved in ethanol, and the hydrochloride formed by the addition of 5N-hydrochloric acid. Sodium chloride, which was precipitated, was filtered off and the ethanol solution evaporated to dryness. Crystallisation of the solid residue from aqueous acetone gave N-3-*amino*-7α : 12α-*dihydroxycholanoylethylenediamine dihydrochloride* (245 mg.) as needles, m. p. 260° (decomp.), $[\alpha]_D^{20}$ +44° (*c*, 1·0 in H_2O) (Found, after drying at 190°: N, 7·9. $C_{26}H_{47}O_3N_3$,2HCl requires N, 8·0%).

Esters of 3-*Amino*-7α : 12α-*dihydroxycholanic Acid Hydrochloride.*—The hydrochloride

TABLE 1. *Alkyl* 3-*amino*-7α : 12α-*dihydroxycholanoate hydrochlorides.*

		Found (%)				Required (%)		
Alkyl	Decomp.	C	H	N	Formula	C	H	N
Me	250°	65·6	9·8	3·15	$C_{25}H_{43}O_4N$,HCl	65·6	9·8	3·1
Et	260—270	66·6	9·6	3·0	$C_{26}H_{45}O_4N$,HCl	66·1	10·0	3·0
Pr^n	235	64·6	9·8	2·8	$C_{27}H_{47}O_4N$,HCl,H_2O	64·6	10·1	2·8
Bu^n	250	65·4	9·6	2·7	$C_{28}H_{49}O_4N$,HCl,H_2O	65·0	10·2	2·7
n-C_5H_{11}	250	65·6	10·2	2·8	$C_{29}H_{51}O_4N$,HCl,H_2O	65·5	10·3	2·65
n-C_6H_{11}	260	65·9	10·3	2·5	$C_{30}H_{53}O_4N$,HCl,H_2O	66·1	10·5	2·6
n-C_8H_{17}	270	67·5	10·7	2·2	$C_{32}H_{57}O_4N$,HCl,H_2O	67·0	10·6	2·4
Pr^i	270	66·6	10·1	3·0	$C_{27}H_{47}O_4N$,HCl	66·7	10·1	2·9
Bu^i	235	67·0	10·2	2·7	$C_{28}H_{49}O_4N$,HCl	67·3	10·2	2·8
CH_2Bu^i	260—270	65·3	10·5	2·7	$C_{29}H_{51}O_4N$,HCl,H_2O	65·5	10·3	2·65
$CH_2{\cdot}CH_2Bu^i$	250	67·5	10·4	2·85	$C_{30}H_{53}O_4N$,HCl	68·3	10·4	2·65
$Bu^n{\cdot}CHEt{\cdot}CH_2$	250	67·3	10·7	2·5	$C_{32}H_{57}O_4N$,HCl,H_2O	67·0	10·6	2·4

TABLE 2. *The bacteriostatic activities of some basic bile acid derivatives.*

No.	Hydrochloride of	Limiting bacteriostatic dilution against: *Staph. aureus* 16 hr. at 37°	*Staph. aureus* 40 hr. at 37°	*Aerobact. aerogenes* 16 hr. at 37°	*Aerobact. aerogenes* 40 hr. at 37°
1	23-Aminonorcholan-3α-ol	256,000	256,000	Inactive	Inactive
2	23-Aminonorcholan-3-one	64,000	64,000	Inactive	Inactive
	Ethylenediamine derivatives				
3	*N*-3α : 7α : 12α-Trihydroxycholanoyl-	8000	8000	2000	2000
4	*N*-3α : 12α-Dihydroxycholanoyl-	32,000	32,000	16,000	16,000
5	*N*-3α-Hydroxycholanoyl-	32,000	16,000	—	—
6	*N*-3α : 12α-Dihydroxychol-8(14)-enoyl-	16,000	16,000	4000	4000
7	*N*-3α : 12α-Dihydroxychol-14(15)-enoyl-	32,000	16,000	4000	2000
8	*N*-3-Amino-7α-12α-dihydroxycholanoyl- (dihydrochloride)	32,000	16,000	Inactive	Inactive
	Esters of 3-amino-7α : 12α-dihydroxycholanoic acid				
9	Methyl	8000	8000	2000	2000
10	Ethyl	16,000	16,000	4000	4000
11	*n*-Propyl	16,000	8000	16,000	8000
12	*n*-Butyl	64,000	32,000	8,000	8000
13	*n*-Pentyl	128,000	128,000	32,000	8000
14	*n*-Hexyl	256,000	256,000	4000	2000
15	*n*-Octyl	128,000	32,000	Inactive	Inactive
16	*iso*Propyl	16,000	16,000	8000	8000
17	*iso*Butyl	32,000	32,000	32,000	16,000
18	*iso*Pentyl	64,000	64,000	32,000	16,000
19	*iso*Hexyl-	8000	4000	Inactive	Inactive
20	2-Ethylhexyl	2000	2000	Inactive	Inactive

TABLE 3. *Depression of the surface tension (dynes/cm.) of nutrient-broth medium.*

No.	Dilution, 1 in.: 2000	4000	8000	16,000	32,000	64,000	128,000	256,000	512,000
1	—	—	—	—	2·7	2·1	1·0	<1	—
2	—	—	—	3·1	1·8	1·1	0·5	—	—
3	8·9	8·7	6·0	4·2	3·3	—	—	—	—
4	—	—	—	5·1	4·2	2·9	2·4	—	—
5	—	—	—	2·5	1·9	1·9	1·6	—	—
6	—	7·1	6·2	5·6	3·2	2·1	1·2	—	—
7	6·5	5·1	4·2	3·3	2·6	2·0	1·4	—	—
8	—	—	3·1	2·5	2·3	1·3	0·5	—	—
9	—	9·6	8·0	6·8	6·3	—	—	—	—
10	—	10·7	8·7	7·8	7·0	6·6	—	—	—
11	—	—	13·2	9·9	10·6	7·0	—	—	—
12	—	—	13·7	12·5	12·2	10·8	8·8	—	—
13	—	—	14·2	16·3	15·5	14·2	11·2	9·3	—
14	—	17·4	18·0	18·0	—	17·1	16·3	13·2	10·7
15	—	—	—	14·5	13·1	12·8	8·0	6·3	4·3
16	—	—	13·7	11·5	10·9	8·2	—	—	—
17	—	—	—	14·2	11·2	8·8	7·7	—	—
18	—	—	—	14·3	13·2	11·6	7·9	—	—
19	—	16·1	14·8	13·6	10·6	—	—	—	—
20	17·5	16·8	15·5	10·5	—	—	—	—	—

At limiting dilution bacteriostatic (after 40 hr.) for:

No.	*Staph. aureus*	*Aerobact. aerogenes*	No.	*Staph. aureus*	*Aerobact. aerogenes*
1	<1	Inactive	11	13·2	13·2
2	1·1	Inactive	12	12·2	13·7
3	6·0	8·9	13	11·2	14·2
4	4·2	5·1	14	13·2	—
5	2·5	—	15	13·1	Inactive
6	5·6	7·1	16	11·5	13·7
7	3·3	5·1	17	11·2	14·2
8	2·5	Inactive	18	11·6	14·3
9	8·0	—	19	16·1	Inactive
10	7·8	10·7	20	17·5	Inactive

(Jones, Webb, and Smith, *J.*, 1949, 2164) was heated on a boiling-water bath with the appropriate alcohol, either containing 20% of dry hydrogen chloride or mixed with 8% of 10N-hydrochloric acid. The solvents were removed by distillation or steam-distillation under reduced pressure. The syrupy *ester hydrochlorides* solidified on trituration with acetone. They crystallised and were recrystallised from ethanol–acetone (1 : 4) or water (see Table 1). Analyses were of samples dried at 60° under reduced pressure : some of the esters then still retained water of crystallisation.

Determination of Bacteriostatic Activity.—The bacteriostatic activities of the various derivatives against *Staphylococcus aureus* and *Aerobacter aerogenes* were determined by the serial-dilution method in a nutrient broth of the following composition : Lab. Lemco 1%, Oxoid bacteriological peptone 1%, and sodium chloride 0·5% (adjusted to pH 7).

Tubes of media containing suitable concentrations of the bile acid derivatives were sterilised for 30 min. at 15 lb./sq. in., cooled, and inoculated from a sterile pipette with one drop of a 24 hr. culture of the organism in nutrient broth. After a suitable time, the inhibition of growth of the organism was measured by comparison with the control. Determinations were in duplicate and are recorded in Table 2. The activities of 23-aminonorcholan-3α-ol hydrochloride and 23-aminonorcholan-3-one hydrochloride (Hilton and Webb, *loc. cit.*) are included for comparison.

Determination of Surface Activity.—The depression of the surface tension of the nutrient-broth medium was determined over a range of concentrations. Surface tension was measured with the du Nöuy tensiometer on 5 ml. of the solution 2 minutes after the formation of the surface (this time was important for reproducibility; see Gaddum, *Proc. Roy. Soc.*, 1931, *B*, **109**, 114). These results are recorded in Table 3.

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