[CONTRIBUTION FROM THE LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY]

7-AMINOSTEROIDS[1, 2]

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During the course of work in this laboratory on the preparation of $\Delta^{5,7}$-steroids (1) by the Wohl-Ziegler method, it was discovered that primary and secondary amines, *e.g.*, piperidine, react metathetically with 7-bromocholesteryl esters.[3]

Considerable attention has been devoted to the problem of purity of 7α-bromocholesteryl benzoate,[4] the key intermediate for the preparation of these amines. In our previous publication (1) it was believed that "bromocholesteryl" benzoate (prepared from cholesteryl benzoate and NBS[5]) consisted primarily of 7α-bromocholesteryl benzoate. This problem has now been resolved, and the results indicate unequivocally that the bromination product is identical in all respects with that obtained by the action of phosphorus tribromide on 7β-hydroxycholesteryl benzoate.[6] In the latter displacement reaction Walden inversion necessarily has taken place to afford the α-configuration at the C_7 position.[7] The previously recorded discrepancy in optical rotatory power may be ascribed to the deteriorating effect of chloroform on the bromocompound. The optical rotation must be determined immediately after solution of the compound. By rapid, careful recrystallization is chemically inert solvents the bromocompounds have been obtained in a very high state of purity. In Table I are listed the physical properties of 7α-bromocholesteryl benzoate. The infrared spectra of the samples of 7α-bromocholesteryl benzoate prepared by the two methods discussed above were identical.

The preparation of 7-aminosteroids may generally be represented as follows, and was carried out under a variety of conditions. Solutions of I in toluene or xylene were reacted with the various amines at reflux temperatures for 10–15 minutes, and gave II. The reaction was carried out also at room-temperature in ether, ethyl acetate, or benzene. In some cases excess amino was used as the solvent. II, in turn, was hydrolyzed with alcoholic potash to afford III. The

[1] Presented before the Division of Organic Chemistry at the 116th meeting of the American Chemical Society, Atlantic City, N. J., September 18 to 23, 1949.

[2] Previous publications on 7-Aminosteroids; Barnett, Ryman, and Smith, *J. Chem. Soc.*, 524 (1946); Eckhardt, *Ber.*, **71**, 469 (1938).

[3] In a recent publication by Bide, Henbest, Jones, Peevers, and Wilkinson, *J. Chem. Soc.*, 1783 (1948), a similar observation was recorded. No experimental details were given.

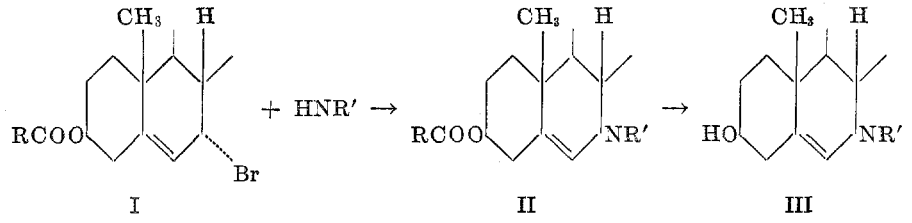
[4] The assignment of the α-configuration for the bromine atom at the C_7-position follows that of Bide, Henbest, Jones, and Wilkinson, *J. Chem. Soc.*, 1788 (1948), and that of Fieser, *Experientia*, **6**, 312 (1950).

[5] NBS = N-Bromosuccinimide.

[6] The assignment of absolute configuration to 7-hydroxysteroids has been discussed by Fieser, Fieser, and Chakravarti, *J. Am. Chem. Soc.*, **71**, 2226 (1949), and by Barton, *J. Chem. Soc.*, 2174 (1949).

[7] That Walden inversion occurred in this reaction has been proposed also by Bide, *et al.*, *loc. cit.*

following aminosteroids were prepared: 7-anilinocholesterol, 7-piperidinocholesterol, 7-(4′-acetaminoanilino)cholesterol, 7-(3′-pyridinoamino)cholesterol, and



I II III

7-(4′-carbethoxypiperazino)cholesterol. In Table II are listed the physical constants of the five aminosteroids and various derivatives.

TABLE I

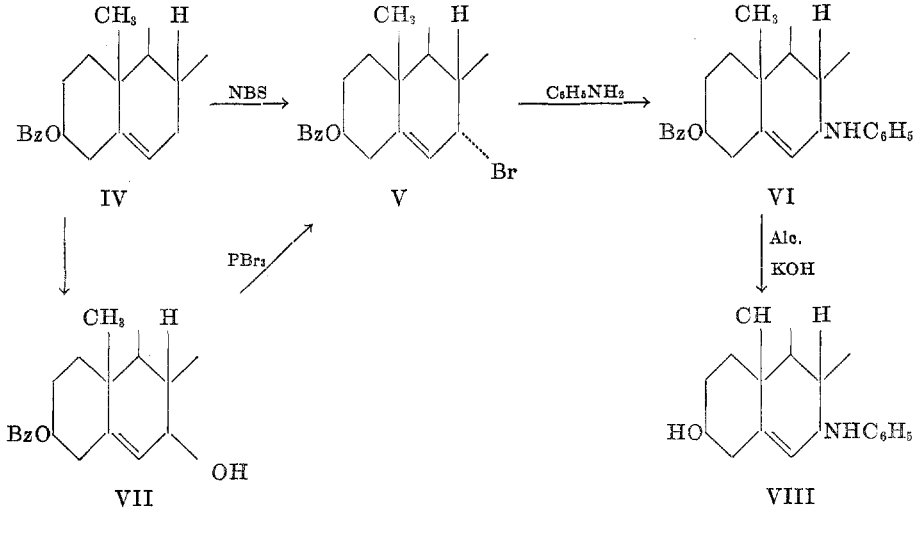
PHYSICAL PROPERTIES OF 7α-BROMOCHOLESTERYL BENZOATE[a]

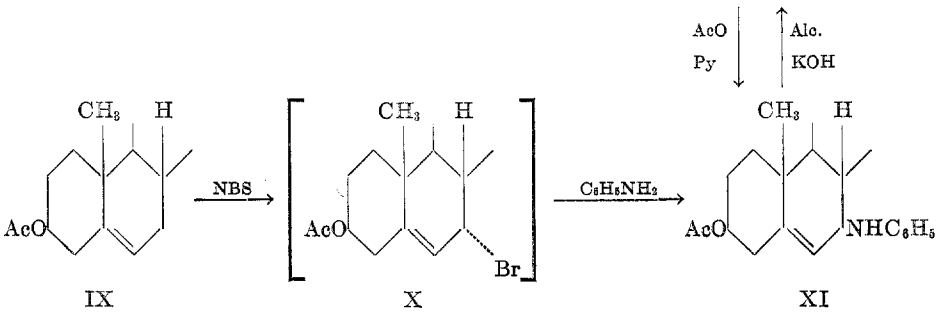
METHOD OF PREPARATION	M.P., °C.	$[\alpha]_D$ ($CHCl_3$)	U. V. ABSORPTION SPECTRUM (ETHER)
Cholesteryl benzoate + NBS	145.8–147 d.	−178.5°	λ_{max} 229 mμ ε = 23,200
7β-Hydroxycholesteryl benzoate + PBr_3	146–146.6 d.	−174.6°	λ_{max} 229 mμ ε = 24,400

[a] See ref. (1–6) for previously recorded data on this compound.

The major part of our work has been centered on 7-anilinocholesterol, and the various preparations and transformations are contained in Flowsheets I and II, which are self-explanatory.

FLOWSHEET I

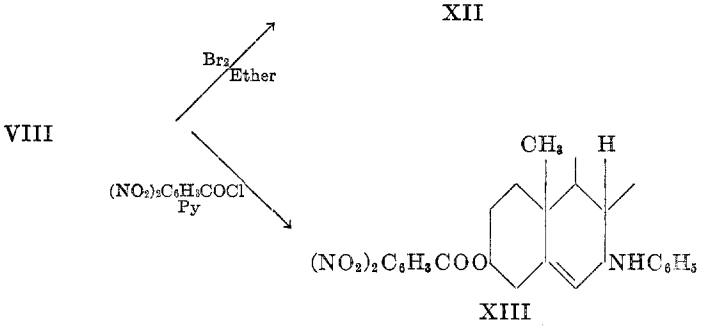


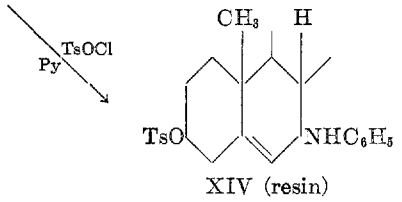


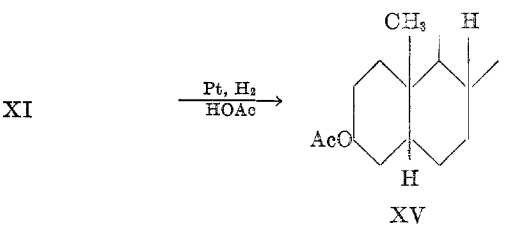
FLOWSHEET II

Tribromo-7-anilinocholesterol

XII





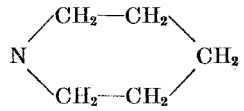
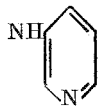
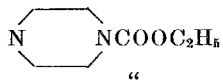


The reaction when extended to 3β,7α-dibromo-Δ^5-cholestene[8] (4, 7) and piperidine gave a resin which analyzed correctly for a piperidinocholesteryl

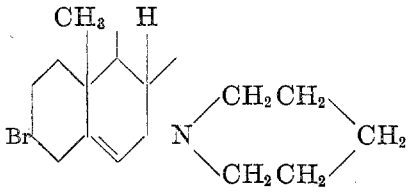
[8] The bromine atom at the C_7-position has been assigned the α-configuration in accord with our belief that NBS bromination of Δ^5-steroids affords the α-bromocompound regardless of the substituent at the C_3-position. This does not necessarily mean that no other products are formed.

TABLE II

7-AMINOSTEROIDS AND DERIVATIVES

COMPOUND	FORMULA	R	NR′	M.P., °C.	$[\alpha]_D$(CHCl$_3$)	ULTRAVIOLET ABSORPTION SPECTRUM MAXIMA, mμ (1% CHLOROFORM-ABSOLUTE ALCOHOL)
7-Anilinocholesterol	III (VIII)	—	NHC_6H_5	161–163	132.6	255, 304–306
7-Anilinocholesteryl acetate	II (XI)	CH_3	NHC_6H_5	185–187	96.7	255, 302–306
7-Anilinocholesteryl benzoate	II (VI)	C_6H_5	NHC_6H_5	187–189	132.0	230–231, 255, 302–306
7-Anilinocholesteryl 3,5-dinitrobenzoate	II (XIII)	$(NO_2)_2C_6H_3$	NHC_6H_5	196.5–198	113	249, 255, 302–305
7-Piperidinocholesterol	III	—		146–150	104.5	None
7-Piperidinocholesteryl benzoate	II	C_6H_5	"	165–167	92.2	229
7-(4′-Acetaminoanilino)cholesterol	III	—	$NHC_6H_4NHCOCH_3(p)$	205–206	147.3	277–278
7-(4′-Acetaminoanilino)cholesteryl benzoate	II	C_6H_5	$NHC_6H_4NHCOCH_3(p)$	223 d.	142.5	229, 275–280
7-(3′Pyridinoamino)cholesterol	III	—		196.5–198	135.8	260, 321–324
7-(3′Pyridinoamino)cholesteryl benzoate	II	C_6H_5	"	225–226.5	139.8	229–230, 259, 320–323
7-(4′-Carbethoxypiperazino)cholesterol	III	—		—	—	None
7-(4′-Carbethoxypiperazino)cholesteryl benzoate	II	C_6H_5	"	134–136	93.2	229.5

bromide to which we assigned the structure XVI. The relative stability of 3-halogenocompounds of this type has been discussed elsewhere (7, 8).



XVI

It has been found that the expected aminosteroid is not obtained in all cases, *e.g.* an attempted preparation with 2-aminopyrimidine. This amine when heated with 7α-bromocholesteryl benzoate in xylene gave principally 7-dehydrocholesteryl benzoate in 29% yields.

TABLE III

C_7-CONFIGURATION

COMPOUND	C-7 CONFIG.	$[\alpha]_D$(CHCl$_3$)
7-Aminocholesterol	β	+181°
	α	−327°
7-Acetamidocholesterol	β	+85°
		+81° (1:1 CHCl$_3$-MeOH)
	α	−183° (1:1 CHCl$_3$-MeOH)
		−194°
7-Anilinocholesterol	β	+133°
7-Piperidinocholesterol	β	+105°
7-(4′-Acetaminoanilino)cholesterol	β	+147°
7-(3′-Pyridinoamino)cholesterol	β	+140°

The free aminosteroids (III), in our hands, did not form sparingly soluble digitonides with digitonin. Also, it was found that 7-anilinocholesterol (VIII) did not form insoluble salts with hydrochloric, sulfuric, phosphoric, or citric acids.

The structure (III) assigned to the aminosteroids is based on the theory of least alteration of structure; there is no unequivocal proof for this. If the proposed structure is assumed to be correct, there remains the problem of assigning a configuration at the C_7-center. In this connection, we have reached the *tentative* conclusion that the grouping is attached in the β-configuration. This speculation is based on the following arguments.

Bide, Henbest, Jones, and Wilkinson (5) have postulated the generalization that *β-7-substituted cholesterol derivatives are dextrorotatory* while the *α-7-compounds are levorotatory*.[9, 10] Epimeric 7-aminosteroids have been prepared by

[9] Bide and co-workers actually stated the generalization with provisional relative configuration of "α" and "β" which are the reverse of the absolute configuration.

[10] Barton, *J. Chem. Soc.*, 2174 (1949) has noted that the 3α,7-dihydroxy-Δ^5-cholestenes do not conform to this generalization.

Barnett, Ryman, and Smith, *loc. cit.;* the rotations of these compounds are given in Table III. On the basis of the wide difference in rotations between the epimeric pairs it appeared reasonable to assume that these compounds obey the generalization of Bide and co-workers. Consequently, the positive rotatory epimers have been assigned the β-configuration, and the negative rotatory, the α-configuration. An examination of the optical rotatory powers of four of the aminosteroids (Table III) reported herein shows them to be all highly positive. Therefore, they have been assigned the β-configuration at the C_7-center. The conclusion is thereby reached that the preparation of aminosteroids from 7α-bromocholesteryl esters proceeded by Walden Inversion.

In Table IV, the aminosteroids have been analyzed according to the Method of Molecular Rotation Differences (9). As was to be expected, substitution at the C_7-position exerts a considerable "vicinal effect" at the C_3-$\Delta^{5,6}$ center of asymmetry (9a, f).

TABLE IV

MOLECULAR ROTATION ANALYSIS

	$[M]_D$(CHCl$_3$)				Δ_1	Δ_2	Δ_3	$\Delta\Delta_1$	$\Delta\Delta_2$	$\Delta\Delta_3$
	Sterol	Acetate	Benzoate	3,5-Dinitrobenzoate						
Cholesterol	−154[a]	−188[a]	−74[a]	−81[b]	−34	80	73	—	—	—
7-Anilinocholesterol	633	502	767	758	−131	134	125	−97	54	52
7-Piperidinocholesterol	490	—	528	—	—	38	—	—	−42	—
7-(4′-Acetaminoanilino)-cholesterol	787	—	909	—	—	122	—	—	42	—
7-(3′Pyridinoamino)cholesterol	650	—	814	—	—	164	—	—	84	—

[a]Reference 9f. [b] Reference 9c.

EXPERIMENTAL

Absorption spectra. All spectra were determined with a Beckman Quartz spectrophotometer (mfg'd. by the National Technical Laboratories, Pasadena, California), and were determined, unless otherwise stated, in 1% chloroform-absolute alcohol, *i.e.* the weighed sample was dissolved in 1 ml. of reagent chloroform and this solution was rapidly diluted to 100 ml. with commercial absolute alcohol.

Melting points. All m.p.'s are uncorrected. When a compound has a cloudy melt, the clearing point is given after the m.p.

Optical rotations. All determinations are for chloroform solution (2 ml.), 1 dcm. semimicro tube.

7-α-Bromocholesteryl benzoate (I). *A.* A sample of "bromocholesteryl" benzoate (prepared from cholesteryl benzoate and NBS), m.p. 144.5–146° d., was recrystallized from petroleum ether (b.p. 64–66°), and melted at 147–149° d., λ_{max}^{ether} 229 mμ, ϵ_{229} 24,400, $[\alpha]_D^{27}$ −170.6° (14.3 mg.; α_D^{27} −1.22°). Recrystallization from petroleum ether (b.p. 64–66°) gave m.p. 147–149° d. (regular 0–250° thermometer), m.p. 145.4–145.7° d. (Anschütz thermometer, sample inserted in bath at 143°); λ_{max}^{ether} 229 mμ, ϵ_{229} 21,700, $[\alpha]_D^{27}$ −150.7° (28 mg.; α_D^{27} −2.11°)

(solution for rotation prepared ½–¾ hour before determination). One more recrystallization from petroleum ether gave m.p. 145.8–147° d. (Anschütz thermometer), λ_{max}^{ether} 229 mμ, ϵ_{229} 23,220, $[\alpha]_D^{28}$ −178.5° (12.1 mg.; α_D^{28} −1.08°); $[M]_D$ −1016.

Anal. Calc'd for $C_{34}H_{49}BrO_2$ (569.65): C, 71.86; H, 8.67; Br, 14.03.
Found: C, 71.81; H, 9.06; Br, 14.42.

B. Phosphorus tribromide (0.2 ml.) was added to a benzene solution (10 ml.) of 7β-hydroxycholesteryl benzoate, (m.p. 188.5–191.5°) (0.77 g.) and the mixture was allowed to stand at room temperature for three hours. The cloudy solution was poured into cold water, and the benzene layer was separated. The extract was washed four times with water, dried with magnesium sulfate, and filtered. Evaporation of the benzene *in vacuo* gave a solid residue. Petroleum ether (b.p. 64–66°) was added, the mixture was cooled, and the crystals were collected, wt. 0.31 g., m.p. 139.5–141.5° d. (Anschütz thermometer), λ_{max}^{ether} 229 mμ, ϵ_{229} 24,500, $[\alpha]_D^{27}$ −152.7° (18.2 mg.; α_D^{27} −1.39°). (Solution for rotation prepared ½–¾ hour before determination). Recrystallization from petroleum ether (b.p. 64–66°) gave m.p. 146–146.6° d. (Anschütz thermometer), λ_{max}^{ether} 229 mμ, ϵ_{229} 24,400, $[\alpha]_D^{28}$ −174.6° (12.6 mg.; α_D^{28} −1.10°); $[M]_D$ −993.

Anal. Calc'd for $C_{34}H_{49}BrO_2$ (569.65): C, 71.86; H, 8.67; Br, 14.03.
Found: C, 71.76; H, 9.18; Br, 14.01.

A mixture m.p. determination with preparation (A) showed no depression.

7-Anilinocholesteryl benzoate (VI). *A.* A solution of 19.5 g. of 7α-bromocholesteryl benzoate (prepared from cholesteryl benzoate and N-bromosuccinimide) in 150 ml. of toluene was treated with 7.5 ml. of aniline. The mixture was refluxed for 15 minutes, cooled, and the solid was separated by filtration through Celite. Evaporation of the filtrate *in vacuo* gave a semi-solid which was dissolved in acetone. Concentration and cooling gave a white solid which was washed with acetone; wt. 7.7 g., m.p. 174.5–179.5°, 181°. Two recrystallizations from acetone gave 4.77 g., m.p. 186–188° (with slight previous softening at 185–186°). One gram of this material was recrystallized once more from acetone, wt. 0.58 g., m.p. 187–189° (with previous softening at 186–187°); λ_{max} 230–231, 255, and 302–305 mμ, $\epsilon_{230-231}$ 18,000, ϵ_{255} 18,600, $\epsilon_{302-305}$ 3,210; $[\alpha]_D^{27}$ + 132° (27.05 mg., α_D^{27} +1.79°); $[M]_D$ 767.

Anal. Calc'd for $C_{40}H_{55}NO_2$ (581.85): C, 82.56; H, 9.53; N, 2.41.
Found: C, 82.54; H, 9.65; N, 2.86, 2.60.

From the mother liquors (benzene extraction, recrystallization from acetone) there was obtained an additional 3.11 g. of product, m.p. 186–188°. The total yield of benzoate was 7.46 g.

B. (*Via* N-Bromosuccinimide method without isolation of intermediate bromocompound). A mixture of 9.8 g. (0.02 mole) of cholesteryl benzoate, 4.28 g. (0.024 mole) of N-bromosuccinimide, and 100 ml. of petroleum ether (b.p. 64–66°, double bond-free) was reacted in the usual manner. The crude bromocompound so obtained was treated with 100 ml. of toluene and 6 ml. of aniline in the above manner, and gave 2.07 g. of VI, m.p. 185–186°(softening at 184–185°). The material was again recrystallized from acetone (positive seeding with material from *A.*), wt. 1.56 g., m.p. 185–187°. The melting point was not depressed by admixture with material from *A.* λ_{max} 230.5–231, 255, and 302–305 mμ, $\epsilon_{230.5-231}$ 17,000, ϵ_{255} 18,000, $\epsilon_{302-305}$ 2,940; $[\alpha]_D^{28}$ + 135.2° (18.2 mg., α_D^{28} + 1.23°); $[M]_D$ 786.

C. A mixture of 2 g. (0.0035 mole) of 7α-bromocholesteryl benzoate (prepared from 7β-hydroxycholesteryl benzoate and phosphorus tribromide), 0.72 ml. (0.0075 mole) of aniline, and 25 ml. of toluene was reacted in the above manner and gave 0.2 g., m.p. 186–188° (softening at 185–186°). A mixture melting point determination with material from preparation *A* gave no depression. λ_{max} 230.5–231, 255, and 301–304 mμ, $\epsilon_{230.5-231}$ 18,000, ϵ_{255} 18,600, $\epsilon_{301-304}$ 3,370. $[\alpha]_D^{27}$ +131° (15.3 mg., α_D^{27} +1.00°); $[M]_D$ 745.

7-Anilinocholesterol (VIII). *A.* A mixture of 6.9 g. of 7-anilinocholesteryl benzoate and 200 ml. of 5% alcoholic potash was refluxed on the steam-bath for 50 minutes. Water (200

ml.) was added, the mixture cooled, and an oil separated, which on being worked solidified. The solid was collected, washed with 50% methanol, water, and 50% methanol; wt. 5.79 g., m.p. 159–162°. Recrystallization from dilute methanol gave 5.16 g., m.p. 161–163.5°. One-half gram of this material was further recrystallized from dilute methanol, wt. 0.47 g., m.p. 161–163°; λ_{max} 255, 304–306 mμ, ϵ_{255} 17,600, $\epsilon_{304-306}$ 3,220; $[\alpha]_D^{27}$ +132.6° (18.4 mg., α_D^{27} +1.22°); $[M]_D$ 633.

Anal. Calc'd for $C_{33}H_{51}NO$ (477.75): C, 82.96; H, 10.76; N, 2.93.
Found: C, 82.74; H, 10.90; N, 3.18.

B. 7-Anilinocholesteryl acetate (200 mg.) (prepared from cholesteryl acetate, NBS, and aniline) was hydrolyzed in the usual manner and gave 145 mg. of VIII, m.p. 160.5–163°. Admixture with preparation *A* gave no depression, m.p. 161–163°; λ_{max} 255, 302–306 mμ, ϵ_{255} 18,000, $\epsilon_{302-306}$ 3,000.

7-Anilinocholesteryl acetate (XI). *A.* 7-Anilinocholesterol (0.5 g.) (prepared by hydrolysis of the 7-anilino-benzoate) in 5 ml. of pyridine was treated in the cold with 0.5 ml. of acetic anhydride. The solution was allowed to stand at room temperature overnight; on the addition of cold dilute hydrochloric acid an oil separated which, when worked, solidified. The solid was washed with water, wt. 0.54 g., m.p. 181–184° (with previous softening). Recrystallization from benzene-methanol, and acetone-methanol gave 0.45 g., m.p. 185–187°; λ_{max} 255, 302–305 mμ, ϵ_{255} 17,350, $\epsilon_{302-305}$ 2,840; $[\alpha]_D^{27}$ +96.7° (27.75 mg., α_D^{27} +1.34°); $[M]_D$ 502.

Anal. Calc'd for $C_{35}H_{53}NO_2$ (519.78): C, 80.87; H, 10.28; N, 2.70.
Found: C, 81.15; H, 10.52; N, 2.75.

B. (*Via* cholesteryl acetate and NBS). Cholesteryl acetate (11 g.) in 100 ml. of petroleum ether (b.p. 64–66°, double bond-free) was reacted in the usual manner for 3 minutes with 5.5 g. of NBS. Aniline (5 ml.) was added to the mixture which was cooled and filtered. The turbid filtrate was evaporated *in vacuo*; the residue was treated with 1.6 ml. of aniline and 100 ml. of toluene. The mixture was refluxed for 10 minutes, cooled, and filtered. Evaporation of the filtrate *in vacuo* gave an oil which was dissolved in acetone and treated with methanol. The mixture gave crystals which were separated and washed with methanol, oily crystals (weight not taken). Recrystallization from acetone-methanol gave 3.87 g., m.p. 173–181°. Three further recrystallizations from acetone-methanol gave 2.01 g., m.p. 185–187.5°. Admixture with preparation *A* gave no depression of melting point. λ_{max} 255, 303–306 mμ, ϵ_{255} 16,800, $\epsilon_{303-306}$ 2,560; $[\alpha]_D^{28}$ +98.2° (17.3 mg., α_D^{28} +0.85°); $[M]_D$ 510.

7-Anilinocholesteryl 3,5-dinitrobenzoate (XIII). 7-Anilinocholesterol (0.5 g.) in 5 ml. of pyridine was reacted in the usual manner with 0.4 g. of 3,5-dinitrobenzoyl chloride and gave 0.55 g. of XIII, m.p. 196.5–198° (red melt) (recrystallization from benzene-absolute alcohol); λ_{max} 249, 255, and 302–305 mμ, ϵ_{249} 26,400, ϵ_{255} 27,000, $\epsilon_{302-305}$ 4,130; $[\alpha]_D$ +113° (24.4 mg., α_D^{27} +1.38°); $[M]_D$ 758.

Anal. Calc'd for $C_{40}H_{53}N_3O_6$ (671.85): C, 71.50; H, 7.95; N, 6.26.
Found: C, 71.76; H, 8.27; N, 6.26.

7-Anilinocholesteryl p-toluenesulfonate (XIV). 7-Anilinocholesterol (0.5 g.) in 5 ml. of pyridine was treated in the cold with 0.5 g. of *p*-toluenesulfonyl chloride and the mixture was allowed to stand at room temperature overnight. The product was worked up in ether in the usual manner. The dried ether solution was evaporated and gave a slightly dark colored oil. Attempts to crystallize the oil from petroleum ether (b.p. 64–66°) and acetone were unsuccessful. The solvents were removed *in vacuo*, and the residue was dried in a high vacuum at room temperature, giving a fluffy glass.

Anal. Calc'd for $C_{40}H_{57}NO_3S$ (631.93): C, 76.02; H, 9.09; N, 2.22; S, 5.07.
Found: C, 75.79; H, 9.33; N, 2.30; S, 5.39.

Tribromo-7-anilinocholesterol. 7-Anilinocholesterol (0.5 g.) in 25 ml. of absolute ether was treated with 0.09 ml. of bromine in 25 ml. of absolute ether. This gave an immediate precipitate, which was collected; wt. 0.35 g., m.p. 108° d.

Anal. Calc'd for $C_{33}H_{50}Br_3NO$ (716.47): C, 55.32; H, 7.03; Br, 33.46.
Found: C, 55.11; H, 7.07; Br, 33.62.

Hydrogenation of 7-anilinocholesteryl acetate. A mixture of 1 g. (0.0019 mole) of 7-anilinocholesteryl acetate, 0.1 g. of platinum oxide, and 75 ml. of glacial acetic acid was shaken in an atmosphere of hydrogen for 24 hours at room temperature (25°). The acetate was initially insoluble, but dissolved on hydrogenation. Approximately 204 ml. (STP) of hydrogen was consumed by the compound as compared with the theoretical values of 189 and 213 ml. for 4 and 5 equivalents resp.

The catalyst was removed and the filtrate was evaporated *in vacuo*. The residue was crystallized from dilute methanol; wt. 0.31 g., m.p. 100–103°. Recrystallization from methanol (plus a few drops of water) raised the m.p. to 104–106°. Two further recrystallizations from methanol gave no change; m.p. 104–105°, wt. 130 mg.

Anal. Calc'd for $C_{29}H_{48}O_2$ (Cholestanyl acetate) (428.67): C, 81.25; H, 11.29; N, 0.
Found: C, 80.67; H, 11.73; N, 0.

Due to the low m.p.,[11] all fractions were combined and rehydrogenated as above (0.05 g. of platinum oxide, 50 ml. of glacial acetic acid). The crude product was crystallized from methanol, wt. 0.14 g., m.p. 100–106°. Five recrystallizations from methanol gave m.p. 108–110°. The infrared spectrum of the material was identical with that of an authentic sample of cholestanyl acetate (XV).

7-(3′-Pyridinoamino)cholesteryl benzoate. A mixture of 4.0 g. 7 α-bromocholesteryl benzoate, 3.76 g. of β-aminopyridine, and 100 ml. of ethyl acetate was allowed to stand at room temperature for two days. The precipitate was washed with benzene. The filtrate and washings were concentrated *in vacuo* and methanol was added. This gave 1.3 g. of product, m.p. 216–219°. Two recrystallizations from benzene-acetone gave 0.4 g., m.p. 225–226.5°; λ_{max} 229–230, 259, and 320–323 mμ., $\epsilon_{229-230}$ 18,200, ϵ_{259} 22,900, $\epsilon_{320-323}$ 3,850; $[\alpha]_D^{27}$ +139.8° (5.15 mg., α_D^{27} +0.36°); $[M]_D$ 814.

Anal. Calc'd for $C_{39}H_{54}N_2O_2$ (582.73): C, 80.38; H, 9.32; N, 4.81.
Found: C, 80.37; H, 9.84; N, 4.96.

7-(3′-Pyridinoamino)cholesterol. Hydrolysis of 2.75 g. of benzoate with alcoholic potash was done in the usual manner. The product was worked up in ether. The extract was washed three times with water. The product began to crystallize after the last wash. The ether mixture was cooled, and the crystals were separated, wt. 1.89 g., m.p. 197.5–199°. The free sterol was recrystallized from acetone-petroleum ether; wt. 1.42 g., m.p. 196.5–198°; λ_{max} 260 and 321–324 mμ, ϵ_{260} 21,800, $\epsilon_{321-324}$ 3,720; $[\alpha]_D^{30}$ +135.8° (22.4 mg., α_D^{30} +1.52°), $[\alpha]_{5461}^{30}$ +169.9° (22.4 mg., α_{5461}^{30} +1.90°); α_{Hg}/α_D 1.25; $[M]_D$ 650.

Anal. Calc'd for $C_{32}H_{50}N_2O$ (479.74): C, 80.28; H, 10.53; N, 5.85.
Found: C, 79.93; H, 10.75; N, 6.22.

7-Piperidinocholesteryl benzoate. A mixture of 15.0 g. of 7 α-bromocholesteryl benzoate, 10 ml. of piperidine, and 150 ml. of toluene was refluxed for 10 minutes, cooled and filtered. The filtrate was evaporated *in vacuo*. Addition of acetone to the residue in a small amount of toluene gave crystals, wt. 9.6 g., m.p. 146–154°. Six recrystallizations from benzene-acetone, and benzene-absolute alcohol gave 1.9 g., m.p. 165–167°; λ_{max} 229 mμ, ϵ_{229} 14,300; $[\alpha]_D^{29}$ +92.2° (14.75 mg., α_D^{29} +0.68°); $[M]_D$ 528.

Anal. Calc'd for $C_{39}H_{59}NO_2$ (573.87): C, 81.62; H, 10.36; N, 2.24.
Found: C, 81.90; H, 10.77; N, 2.48.

7-Piperidinocholesterol. To a solution of 5 g. of potassium hydroxide in 100 ml. of alcohol was added 13.1 g. of 7-piperidinocholesteryl benzoate. The mixture was refluxed for 1.5 hours, cooled, and filtered. The crystals so obtained were washed successively with methanol, water, and methanol; wt. 9.9 g., m.p. 150–152°. Recrystallization from alcohol gave 9.2 g., m.p. 146–150°. The free sterol showed no absorption maxima in the ultraviolet. $[\alpha]_D^{27}$ +104.5° (23.7 mg., α_D^{27} +1.24°); $[M]_D$ 490.

Anal. Calc'd for $C_{32}H_{55}NO$ (469.77): C, 81.81; H, 11.80; N, 2.98.
Found: C, 81.86; H, 12.19; N, 3.22.

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[11] Heath-Brown, *et al.*, *J. Chem. Soc.*, 1482 (1940), have recorded the m.p. 109–110° for cholestanyl acetate (XV).

From the mother liquors there was obtained 2.2 g., m.p. 150–152°. Recrystallization from absolute alcohol gave 1.44 g., m.p. 150–153° (m.p. taken after a water wash of the crystals; this treatment appeared to affect the melting point).

7-Piperidinocholesteryl bromide. A mixture of 3 g. of 7 α-bromocholesteryl bromide and 2 ml. of piperidine in 30 ml. of toluene was refluxed for 15 minutes, cooled, treated with anhydrous magnesium sulfate, and filtered through Celite. Evaporation of the filtrate *in vacuo* gave an oil which was dissolved in ether. The solution was washed successively with water, dilute hydrochloric acid, water, sodium bicarbonate, and water. The ether extract was dried with magnesium sulfate, treated with Norit, and filtered. Concentration of the ether solution with simultaneous addition of ethanol, until only ethanol remained, gave an oil which on being cooled gave a mixture of a glass and solid. It was filtered and washed with alcohol, wt. 0.33 g.

Anal. Calc'd for $C_{32}H_{54}BrN$ (532.68): C, 72.15; H, 10.22; N, 2.63; Br, 15.00.

Found: C, 72.25; H, 10.20; N, 2.93; Br, 14.46.

7-(4′-Acetaminoanilino)cholesteryl benzoate. A mixture of 39.6 g. (0.069 mole) of 7 α-bromocholesteryl benzoate, 22.0 g. (0.146 mole) of *p*-aminoacetanilide, and 200 ml. of toluene was refluxed for 10 minutes, cooled and filtered. The filtrate was evaporated *in vacuo*. The residue was dissolved in acetone, and absolute alcohol was added. This gave 7.3 g. of material, m.p. 125° unsharp. Recrystallization from acetone gave 2.4 g. of impure 7-dehydrocholesteryl benzoate, m.p. 132–137°, 169°.

Triangular recrystallization of the mother liquors from chloroform-methanol gave 2.15 g. of the desired product, m.p. 223° d.; λ_{max} 229, 275–280 mμ, ϵ_{229} 18,110, $\epsilon_{275-280}$ 21,050; $[\alpha]_D^{27}$ +142.5° (23.3 mg., α_D^{27} +1.66°); $[M]_D$ 909.

Anal. Calc'd for $C_{42}H_{58}N_2O_3$ (638.90): C, 78.95; H, 9.15; N, 4.38.

Found: C, 79.10; H, 9.33; N, 4.49.

7-(4′-Carbethoxy)piperazinocholesteryl benzoate. A solution of 20 g. of 7 α-bromocholesteryl benzoate in100 ml. of benzene was refluxed with 15 ml. of N-carbethoxypiperazine for 1 hour. The mixture was filtered hot and the filtrate was evaporated *in vacuo*. The residue was dissolved in propanol-2; addition of water gave an oil. The oil was separated by decantation and upon addition of acetone began to crystallize. Addition of alcohol promoted crystallization, wt. 5.2 g., m.p. 125–135°. Two recrystallizations from acetone-methanol gave 3.75 g., m.p. 134–136°; λ_{max} 229.5 mμ, $\epsilon_{229.5}$ 17,100; $[\alpha]_D^{30}$ +93.2°, $[\alpha]_{Hg}^{30}$ +113.9°, α_{Hg}/α_D 1.22; $[M]_D$ 602 (28.1 mg., α_D^{30} +1.31°, α_{Hg}^{30} +1.60°).

Anal. Calc'd for $C_{41}H_{66}N_2O_4$ (646.93): C, 76.11; H, 9.66; N, 4.33.

Found: C, 76.07; H, 9.73; N, 4.43.

7-(4′-Carbethoxy)piperazinocholesterol. To 13 g. of potassium hydroxide in 250 ml. of alcohol was added 34 g. of 7-(4′-carbethoxypiperazino)cholesteryl benzoate. The mixture was refluxed for 1 hour, cooled, and diluted with water. The product was worked up in ether, and the extract was washed with water, dried, and evaporated. This gave an odoriferous oil which on standing set to a glass. As the glass analyzed low on carbon (*ca.* 1.3%) it was dissolved in 300 ml. of petroleum ether (b.p. 66–68°). The solution was extracted successively with three 165-ml. portions of 50% methanol, three 165-ml. portions of 80% methanol, and three 165-ml. portions of 90% methanol. The petroleum ether solution was dried and evaporated. This gave 15.0 g. of a glass which showed no maximum in the ultraviolet.

Anal. Calc'd for $C_{34}H_{58}N_2O_2$ (542.82): C, 75.23; H, 10.77; N, 5.16.

Found: C, 75.44; H, 11.11; N, 5.23.

Reaction between 7 α-bromocholesteryl benzoate and 2-aminopyrimidine. A mixture of 4 g. (0.007 mole) of 7 α-bromocholesteryl benzoate and 1.9 g. (0.02 mole) of 2-aminopyrimidine in 100 ml. of xylene was refluxed for 15 minutes, cooled and filtered. The filtrate was evaporated *in vacuo* and gave a mixture of oil and solid. Acetone was added, and the crystals were separated; wt. 1.75 g., m.p. 131–136°, 155°. Three recrystallizations from acetone gave 1.0 g. of pure 7-dehydrocholesteryl benzoate, m.p. 140–142.5°, 187°; 29% yield.

From the mother liquor there was obtained 0.13 g. of material, m.p. 121–125.5° (release of solvent at 113°?), λ_{max} 239 mμ. This product was apparently $\Delta^{4,6}$-cholestadienyl benzoate.

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SUMMARY

1. 7α-Bromocholesteryl benzoate has been rigorously characterized.

2. The reaction between 7α-bromocholesteryl esters and primary and secondary amines has been investigated.

3. The following 7-aminosteroids have been prepared: 7-anilinocholesterol, 7-piperidinocholesterol, 7-(4′-acetaminoanilino)cholesterol, 7-(3′-pyridinoamino)-cholesterol, and 7-(4′-carbethoxypiperazino)cholesterol.

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