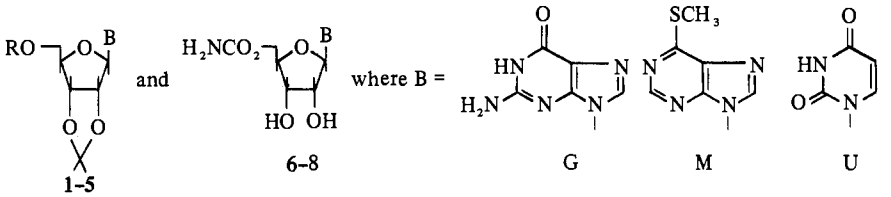
Table I. Nucleoside 5'-*O*-Carbamates and Intermediates[a]



No.	B	R	Mp, °C[a] (solvent)	Yield, %	Analyses
1	G	C_6H_5OCO	263-265 (Me_2CO-CH_3OH)[b]	78	C, H, N
2	G	H_2NCO	290-292 (CH_3OH)[b]	76	C, H, N
3	M	H	105-107 (CH_2Cl_2-C_6H_{14})	74	C, H, N, S
4	U	C_6H_5OCO	61-66 (C_6H_{14})[b]	95	
5	U	H_2NCO	178-179 (C_6H_6-CH_3OH)	99	C, H, N
6	G[c]		253-254 (H_2O)	47	C, H, N
7	M[d]		137-138 (C_2H_5OH)	74[e]	C, H, N, S
8	U		149-150 (C_6H_6-CH_3OH)	79	C, H, N

[a]Melting points (uncorrected) were taken on a Fisher-Johns block. [b]Residue after trituration. [c]$[\alpha]^{22}D$ −42.2° (*c* 1.0, DMF). [d]$[\alpha]^{22}D$ −55.1° (*c* 1.0, DMF). [e]Overall yield from **3**.

residue was washed with acetone (50 ml). Calcium hydroxide (1.0 g) was added to the filtrate and the suspension was stirred for 1 hr. The solid was filtered out and the solution was evaporated to leave a clear oil. Crystallization from methylene chloride–hexane gave 1.68 g (74%) of white crystals, mp 105–107°.

2',3'-Isopropylidine-5'-*O*-carbophenoxyuridine (4). A suspension of 2',3'-isopropylidineuridine (2.0 g, 7.0 mmol) in pyridine (40 ml) was cooled in ice, and phenyl chloroformate (1.0 ml, 8.3 mmol) was added dropwise with stirring. The solution was stirred in ice for 1 hr and then at room temperature for 1 hr. The solvent was stripped and the residue was treated with methanol (40 ml). The solution was again stripped and the clear residue was triturated with two 40-ml portions of water. The residue was dried by stripping with 1:1 methanol–benzene (80 ml) and then triturated with two 40-ml portions of hot hexane. The residue was dissolved in 1:1 acetone–hexane and stripped to leave a white foam. Trituration with cold hexane gave a white solid, mp 61–66° (2.71 g, 95%), homogeneous by tlc with ethyl acetate on silica gel. This material is satisfactory for the next step.

2',3'-Isopropylidine-5'-*O*-carbamoyluridine (5). A solution of 2',3'-isopropylidine 5'-*O*-carbophenoxyuridine (0.5 g, 1.24 mmol) in methanol (5 ml) and concentrated ammonium hydroxide (15 ml) was stirred at room temperature for 2 hr. Evaporation to dryness left a white foam which was triturated with two 25-ml portions of hot 1:1 benzene–hexane. The oil that remained was dissolved in acetone, filtered, and stripped to leave a white foam. Trituration with hexane gave 0.4 g (99%) of white solid melting at 50–80° and homogeneous by tlc (ethyl acetate).

5'-*O*-Carbamoyluridine (8). A solution of 2',3'-isopropylidine-5'-*O*-carbamoyluridine (2.0 g, 6.1 mmol) in 90% trifluoroacetic acid (40 ml) was left at room temperature for 5 min and then stripped to dryness. The residue was dissolved in ethanol (80 ml) and again stripped to leave a clear gum. Crystallization from 1-propanol gave 1.47 g (70%) of white solid melting at 78–82° and homogeneous by tlc (4:1 ethyl acetate–methanol), $[\alpha]^{22}D$ −0.7° (*c* 1.0, water).

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Hypocholesterolemic Agents. 10.[1] Synthesis of Some Model Azacholanic Acids as Potential Regulators of Steroid Biosynthesis and Metabolism†

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Received August 29, 1972

The possible role of cholesterol and other lipids in the etiology of atherosclerosis has focused considerable attention on agents capable of inhibiting cholesterol biosynthesis. One of the physiological sites of regulation in this biosynthesis of cholesterol is thought to be the negative feedback inhibition by cholesterol itself on hydroxymethylglutaryl-CoA reductase, the first irreversible step in this biosynthesis.[2] Previous papers in this series have described the synthesis and potent hypocholesterolemic properties of certain aza- and diazacholesterols which were prepared in an effort to simulate cholesterol in this feedback mechanism. Not only have these compounds been shown to inhibit cholesetrol synthesis in laboratory animals[3] and man,[4] but their ability to interfere with cholesterolgenesis in insects has also been observed.[5]

Since bile acids are end metabolites of cholesterol which also appear to confer a degree of control on cholesterol biosynthesis and are intimately involved in the balance of lipid absorption and excretion in the intestine,[6] it became of interest to examine some model azacholenic acids as potential regulators of steroid biosynthesis and metabolism. This paper represents a continuation of our studies on aza steroids as potential regulators of steroid metabolism and describes the synthesis and biological activities of a number of azacholenic acids.

The most convenient route to C-20 azacholenic and aza-

†This work was supported by Grants HE-11274 from the National Institutes of Health and PRA-18 from the American Cancer Society.

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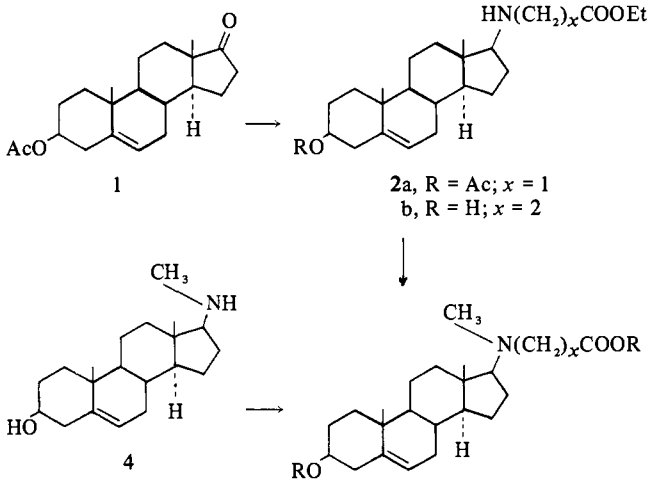
norcholenic acids appeared to be Schiff base formation of the appropriate amino acid ethyl ester with dehydroepiandrosterone acetate (**1**), metal hydride reduction of the intermediate imines to give **2a** or **2b**, and subsequent methylation of the C-20 nitrogens to give 3a or **3c**. Hydrolysis of the latter would then lead to the desired amino acid steroids **3b** and **3d**. In the norcholenic acid series (**3b**) this route gave reasonable yields. Methylation of **2a** was effected by condensation with formalin and metal hydride reduction. Chromatography gave 3a which was hydrolyzed to the corresponding amino acid **3b**. In the azacholenic acid series (**3d**), however, an alternate synthesis of the desired amino acid **3d** was necessary since methylation of **2b** as described above gave unsatisfactory yields of desired product **3c**. An alternate and preferred method consisted of Michael addition of ethyl acrylate to *N*-methyl-17β-aminoandrost-5-en-3β-ol (**4**) to give **3c** and subsequent hydrolysis to the azacholenic acid **3d**. In a similar fashion **3a** could also be obtained by N-alkylation of the amine **4** with ethyl chloroacetate. Subsequent hydrolysis of **3a** again gave the amino acid **3b** (Scheme I).

Scheme I



1

2a, R = Ac; x = 1
b, R = H; x = 2

4

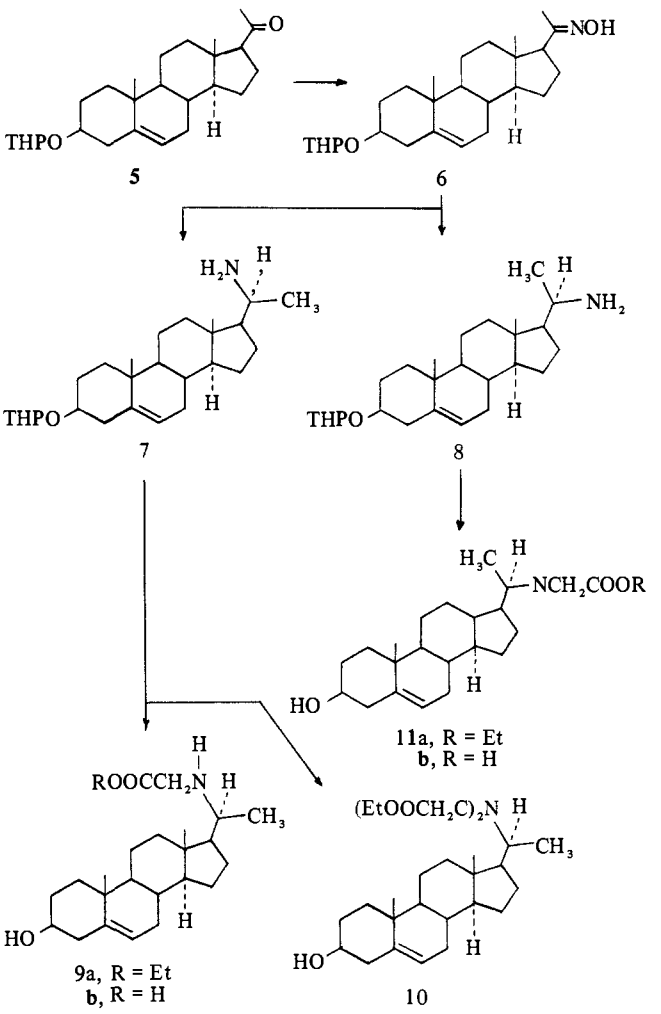
3a, R = Ac; x = 1; R′ = Et
b, R = H; x = 1; R′ = H
c, R = H; x = 2; R′ = Et
d, R = H; x = 2; R′ = H

The epimeric C-22 azacholenic acids were prepared from the readily available 3β-tetrahydropyranyloxypregn-5-en-20-one (**5**). Oxime formation of **5**, followed by reduction with Na in *n*-PrOH, gave a 1:1 mixture of the two isomeric 20-amines **7** and **8** as reported elsewhere.[7] Alkylation of the 20β-amine **7** with 1.1 equiv of ethyl chloroacetate, followed by removal of the protecting group and chromatography, afforded the monoalkylated product **9a** which was hydrolyzed to the desired amino acid **9b**. The dialkylated product **10** was obtained when **7** was treated with an excess of ethyl chloroacetate.

Alkylation of the 20α-amine **8** proved to be more difficult. Treatment of **8** with an excess of ethyl chloroacetate resulted in product mixtures from which neither the mono- or dialkylated products were isolated. However, the monoalkylated product **11a** could be obtained by treatment of **8** with 1 equiv of ethyl chloroacetate. Alkaline hydrolysis of **11a** gave the corresponding 20α-amino acid **11b** (Scheme II).

Of the compounds tested only **2b** (10 mg/kg) has shown oral hypocholesterolemic activity (14%) as determined in rats made hypocholesterolemic with 6-propylthiouracil.[3,7] Its potency, however, was much less than 20,25-diazacholesterol.§ In addition, preliminary *in vivo* studies with the tobacco hornworm (Manduca sexta) have shown **3d** to be less active than its analogous 23-carbon steroidal acid[8] as an inhibitor of Δ^{24}-sterol reductase.#

Scheme II



5

6

7

8

11a, R = Et
b, R = H

9a, R = Et
b, R = H

10

Experimental Section**

Ethyl 3β-Acetoxy-20-aza-21,24-dinorchol-5-en-23-oate (2a). To a solution of dehydroepiandrosterone acetate (**1**, 4 g) in dry C_6H_6 (75 ml) were added freshly prepared glycine ethyl ester (3 ml) and TsOH (100 mg), and the reaction mixture was refluxed for 2 hr with a Dean-Stark apparatus. The reaction mixture was cooled and the solvent was evaporated. The crude imine was dissolved in MeOH (20 ml), $NaBH_4$ (2 g) was added in portions with external cooling, and the reaction mixture was allowed to stir 10 min. Excess H_2O was added to the reaction mixture and the precipitate thus formed was filtered, air-dried, and recrystallized from $(Me)_2CO$ to give 2a (3 g): mp 110–112°; $[\alpha]D$ −44°. *Anal.* ($C_{25}H_{39}NO_4$) C, H.

§We are grateful to Drs. R. E. Ranney and P. D. Klimstra of G. D. Searle and Co. for providing us with this information.

#We are grateful to Dr. James Svoboda, Department of Agriculture, for informing us of this preliminary data.

Satisfactory ir (Perkin-Elmer 337) and nmr (Varian A-60A) spectra were obtained for all compounds reported with the exception of acids **3b, **3d**, **9b**, and **11b** which were not sufficiently soluble for nmr analysis. The melting points were obtained on a Fisher-Johns apparatus and are not corrected. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within 0.4% of the theoretical values. Analyses were performed by Spang Microanalytical Lab, Ann Arbor, Mich., and Midwest Microlab, Ltd, Indianapolis, Ind. Optical rotations were measured in $CHCl_3$, unless indicated otherwise, on a Perkin-Elmer 141 polarimeter at ambient temperature. Mass spectra were taken on a Du Pont 21-490 mass spectrometer.

Ethyl 3β-Acetoxy-20-aza-24-norchol-5-en-23-oate (3a). Formalin (0.6 ml) was added to a solution of **2a** (0.5 g) in MeOH (10 ml) and the mixture stirred for 12 hr. $NaBH_4$ (200 mg) was added to the externally cooled reaction mixture and the contents were allowed to stir 10–15 min before being poured into H_2O. The resulting precipitate was filtered and air-dried. Tlc examination of this material revealed that starting material was still present along with other minor products and the mixture was chromatographed on alumina (Woelm neutral, activity II). Elution with $CHCl_3$–hexane (3:7) afforded the desired compound 3a (300 mg): mp 79–80°; [α]D −69° *Anal.* ($C_{26}H_{41}NO_4$) C, H.

Ethyl 20-Aza-3β-hydroxy-21-norchol-5-en-24-oate (2b). Dehydroepiandrosterone acetate (1, 25 g) was treated with ethyl β-aminopropionate (22.5 g) as in the procedure for **2a**. Reduction with $NaBH_4$ (2.7 g) gave a semisolid which resisted crystallization from common solvents and was hydrolyzed directly to the acid with 10% ethanolic Na_2CO_3. Resterification of a portion of the crude acid (5 g) in 1% ethanolic HCl gave **2b** (3 g): mp 75.5° (Et_2O–*n*-hexane); [α]D −24°. *Anal.* ($C_{24}H_{39}NO_3$) C, H.

20-Aza-24-norchol-5-en-23-oic Acid (3b). A. Hydrolysis of 3a (250 mg) with 5% ethanolic KOH and recrystallization from EtOH gave 3b (200 mg): mp 278–280°; *m/e* 361. *Anal.* ($C_{22}H_{35}NO_3 \cdot 0.5H_2O$) C, H.

B. To a solution of *N*-methyl-17β-aminoandrost-5-en-3β-ol[9] (4, 1 g) in Me_3CN–C_6H_6 (5:1, 50 ml) were added ethyl chloroacetate (0.3 ml), NaI (0.2 g), and Et_3N (0.3 ml), and the mixture was refluxed for 1 hr. The reaction mixture was allowed to cool, poured into H_2O, and extracted with $CHCl_3$. The residue obtained after removal of the solvents *in vacuo* was hydrolyzed with 5% methanolic KOH overnight and made acidic (pH 1), and the HCl salt of 3b was filtered. Distilled H_2O was then added to the HCl salt, the solution was made basic with NH_4OH, boiled until neutral, and cooled, and the precipitate was filtered. Recrystallization from EtOH gave 3b, mp 276–278°, whose ir spectrum was identical with that obtained in method A above.

Ethyl 20-Aza-3β-hydroxychol-5-en-24-oate (3c). To a solution of *N*-methyl-17β-aminoandrost-5-en-3β-ol[9] (4, 7 g) in absolute EtOH (300 ml) was added ethyl acrylate (2.32 g) and the mixture was allowed to stand in the dark for 7 days. Removal of the solvent *in vacuo* gave an amorphous solid. A portion (2 g) was chromatographed on silica gel in order to remove a small amount of starting material. Elution with EtOAc–C_6H_6 (1:9) gave the desired material 3c (1 g): mp 91–92°; [α]D −35°. *Anal.* ($C_{25}H_{41}NO_3$) C, H.

20-Aza-3β-hydroxychol-5-en-24-oic Acid (3d). Hydrolysis of 3c (4 g) with 10% Na_2CO_3 and work-up as indicated for **3b** (method B) gave 3d (1.2 g): mp 208–210° (H_2O–MeOH); [α]D −44° (MeOH). *Anal.* ($C_{23}H_{37}NO_3$) C, H.

Ethyl (20*S*)-3β-Hydroxy-22-azachol-5-en-24-oate (9a). To a solution of the 20β-amine[10] 7 (2 g) in MeCN (75 ml) were added NaI (800 mg), ethyl chloroacetate (0.4 ml), and Et_3N (0.4 ml). After the mixture had refluxed for 1 hr, it was poured into dilute HCl (10%, 200 ml) in order to hydrolyze the THP ether. The mixture was then extracted with $CHCl_3$, dried (Na_2SO_4), evaporated, and chromatographed on alumina (Woelm neutral, activity II). Elution with hexane–$CHCl_3$ (8:2) (300 ml) gave the desired compound **9a** (1 g): mp 110–112° (hexane); [α]D −59°. *Anal.* ($C_{25}H_{41}NO_3$) C, H.

(20*S*)-3β-Hydroxy-22-azachol-5-en-24-oic Acid (9b). Hydrolysis of **10a** with methanolic KOH and the usual work-up gave the 20β-amino acid 9b, mp 284–286°. *Anal.* ($C_{23}H_{37}NO_3$) C, H.

***N,N*-Bis(carboethoxymethyl)-20β-aminopregn-5-en-3β-ol (10).** Treatment of the 20β-amine 7 (250 mg) with excess ethyl chloroacetate (1 ml), NaI (200 mg), and Et_3N (0.5 ml) in a MeCN solution (20 ml) and use of a work-up similar to **9a** afforded the dialkylated product **10** (150 mg): mp 158–160° (MeOH); [α]D −52°. *Anal.* ($C_{29}H_{47}NO_5$) C, H.

Ethyl (20*R*)-3β-Hydroxy-22-azachol-5-en-24-oate (11a). To a solution of the 20α-amine **8** (1 g) in MeCN–C_6H_6 (1:1, 50 ml) were added ethyl chloroacetate (0.4 ml), NaI (0.3 g), and Et_3N (0.3 ml), and the mixture was refluxed for 30 min, cooled, and poured into H_2O. The oily suspension was extracted into $CHCl_3$ and washed with H_2O. The organic layer was evaporated and the residue redissolved in ethanolic HCl (3%, 10 ml) and left at room temperature overnight. The mixture was then neutralized with dilute NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated and the residue was chromatographed on alumina (Woelm neutral, activity II). Elution with petroleum ether (bp 30–60°)–$CHCl_3$ (1:1, 200 ml) gave the desired compound **11a** (150 mg): mp 62–64° (MeOH); [α]D −17°; *m/e* 403. *Anal.* ($C_{25}H_{41}NO_3 \cdot 0.5H_2O$) C, H. Further elution with $CHCl_3$ gave yellow oils which were discarded due to the complexity of the tlc examination.

(20*R*)-3β-Hydroxy-22-azachol-5-en-24-oic Acid (11b). Hydrolysis of **11a** with methanolic KOH and the usual work-up gave the 20α-amino acid **11b**, mp 260–262° (EtOH). *Anal.* ($C_{23}H_{37}NO_3$) C, H.

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Polyethylene Glycol Derivatives of Procaine

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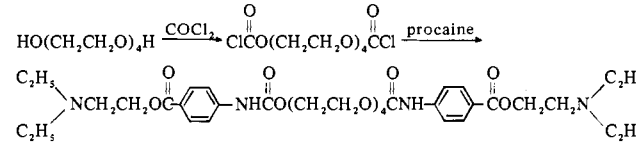
Received September 13, 1972

Recently much interest was manifested in synthetic polymers having biological activity as a means of increasing the duration of activity of drugs.[1-3] Synthetic polymers were used not only as carriers for the drugs but also for their biological activity as a whole.[4-6]

In the present work we studied the attachment of procaine hydrochloride, which is a well-known local anesthetic, to polyethylene glycols as a possible means of prolonging its activity. Previous approaches toward changing the duration of anesthesia of procaine-like compounds were based on carrying out changes in the molecular skeleton, such as increase of the aminoalkyl group, or the intermediate alkylene chain[7-9] and the introduction of alkyl groups into the 4-amino group, which provided prolonged duration of local anesthesia.[10-12]

We chose polyethylene glycols as the carrier polymers because they are known to be nontoxic,[13-16] are soluble in both water and organic solvents, and are available in various well-defined molecular weights.

Use was made of the two terminal hydroxyl groups for attaching the procaine to the polymer. These groups were converted to the corresponding chlorocarbonates, by reaction with phosgene in toluene, which on reaction with procaine gave polyethylene glycols having the procaine attached by carbamate linkages as follows.



The chlorocarbonates were freshly prepared before every reaction, due to slow decomposition. The polyethylene glycol derivatives of procaine were oils which dissolved in solvents