Derivatives of 17a-Aza-D-homo-5-androsten-3β-ol

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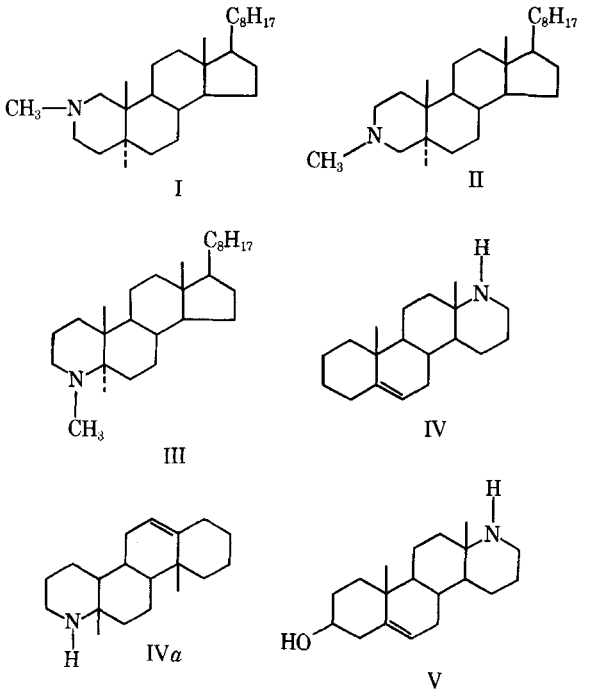
Abstract □ The synthesis of several types of *N*-substituted derivatives of 17a-aza-D-homo-5-androsten-3β-ol as potential antimicrobial agents is reported. Methyl and propyl groups were added by reductive alkylation, and β-dialkylaminoethyl and γ-dialkylaminopropyl groups were introduced by the reaction of dimethylamine or piperidine with the appropriate 17a-(ω-chloroacyl) derivative of VI. None of these derivatives exhibited significant antibacterial or antifungal activity.

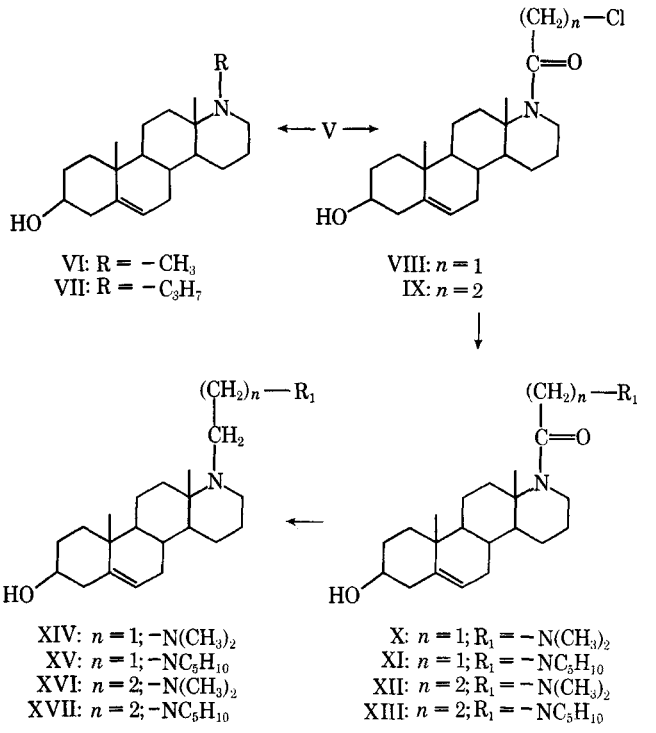
Keyphrases □ 17a-Aza-D-homo-5-androsten-3β-ol derivatives—synthesis, screening as possible antimicrobial agents □ Azasteroids—synthesis, pharmacological evaluation of 17a-aza-D-homo-5-androsten-3β-ol derivatives □ Antimicrobial agents, potential—synthesis, pharmacological screening, 17a-aza-D-homo-5-androsten-3β-ol derivatives

Earlier investigations (1–6) in this laboratory established that certain 4-azasteroids possessed substantial antimicrobial activity. Active derivatives inhibit the growth of most Gram-positive bacteria, molds, and yeasts at concentrations of 0.1–10 mcg./ml. and kill at higher concentrations. More recently, *N*-methyl-2-aza-5α-cholestane (I) and *N*-methyl-3-aza-5α-cholestane (II) were reported to possess antimicrobial properties similar to *N*-methyl-4-aza-5α-cholestane (III) (7). Thus, it appears that 2-azasteroids and 3-azasteroids have antimicrobial properties similar to 4-azasteroids.



This investigation was begun with the objective of determining if 17a-aza-D-homosteroids also possess antimicrobial properties. 17a-Aza-D-homo-5-androstene (IV*a*) resembles a 4-azasteroid in shape and location of nitrogen, as illustrated when its structure is drawn in the unconventional manner (IV*b*). Since the ring fusions are *trans*, the overall shapes are similar. The double bond at position 5 in IV does not produce a major change in the shape of the steroid molecule.

This paper describes the synthesis and preliminary antimicrobial study of derivatives of 17a-aza-D-homo-5-androsten-3β-ol (V). V was prepared in four synthetic steps from androstenolone, as previously described (8–10). 17a-Methyl-17a-aza-D-homo-5-androsten-3β-ol (VI) was prepared by the Leuckart reaction (Method A) or reductive methylation (Method B) of V. The yields were 72% by each method. 17a-Propyl-17a-aza-D-homo-5-androsten-3β-ol (VII) was synthesized in 75% yield by reductive alkylation of V using propionaldehyde (Scheme I).



Scheme I

β-Dialkylaminoethyl (XIV and XV) and γ-dialkylaminopropyl (XVI and XVII) substituents were introduced at position 17a in V through the 17a-(ω-chloroacyl) derivatives (VIII and IX) by reaction with dimethylamine or piperidine, in the presence of alkali, and subsequent reduction of the resulting amides with lithium aluminum hydride. Representative synthetic procedures are described in the *Experimental* section, and data on these steroids are presented in the *Experimental* section and Table I.

Azasteroids V, VI, VII, XIV, XV, XVI, and XVII were screened by serial dilution (1) for antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*,

Table I—*N*-Substituted Derivatives of 17a-Aza-D-homo-5-androsten-3β-ol

Compound	R	Method	Recrystallization Solvent[a]	Yield, %	Melting Point	Molecular Formula	Analysis, % Calc.	Analysis, % Found
VII	C_3H_7	B	Ac	75	308–309°	$C_{22}H_{38}BrNO$[b,c]	C, 64.06 H, 9.28 N, 3.39	C, 64.60 H, 9.31 N, 3.32
XI	$COCH_2NC_5H_{10}$	C		70	302–303°	$C_{26}H_{43}BrN_2O_2$[b,d]	C, 63.01 H, 8.75 N, 5.65	C, 62.87 H, 8.66 N, 5.84
XV	$(CH_2)_2NC_5H_{10}$	D	Et–W	95	123–125°	$C_{26}H_{44}N_2O$	C, 77.95 H, 11.07 N, 6.99	C, 77.69 H, 11.02 N, 6.90
						$C_{38}H_{50}N_8O_{15}$[e]	C, 53.15 H, 5.86 N, 13.04	C, 53.19 H, 5.72 N, 12.89
XII	$CO(CH_2)_2N(CH_3)_2$	C	Et	89	210–212°	$C_{24}H_{40}N_2O_2$	C, 74.18 H, 10.38 N, 7.21	C, 74.32 H, 10.56 N, 7.24
					233–234°	$C_{30}H_{43}N_5O_9$[e]	C, 58.33 H, 7.02 N, 11.34	C, 58.40 H, 7.08 N, 11.49
XVI	$(CH_2)_3N(CH_3)_2$	D	Et	88	146–147.5°	$C_{24}H_{42}N_2O$	C, 76.95 H, 11.30 N, 7.48	C, 76.99 H, 11.32 N, 7.21
XVII	$(CH_2)_3NC_5H_{10}$[f]	D	Et–W	75	156–158°	$C_{27}H_{46}N_2O$	C, 78.21 H, 11.18 H, 6.76	C, 78.39 H, 11.40 H, 6.84
					227–229°	$C_{38}H_{50}N_8O_{15}$[e]	C, 53.67 H, 6.00 N, 12.83	C, 53.06 H, 5.79 N, 12.67

[a] Ac = acetone; Et = ethanol; W = water. [b] HBr salt. [c] Calc. for Br: 19.38. Found: 19.25. [d] Calc. for Br: 16.31. Found: 15.91. [e] Picrate salt. [f] Intermediate XIII, prepared by Method C, was used without complete purification.

Aspergillus niger, and *Candida albicans*. None of these azasteroids exhibited activity at concentrations as high as 100 mcg./ml. and, hence, are considered inactive.

EXPERIMENTAL

17a-Methyl-17a-aza-D-homo-5-androsten-3β-ol (VI)—*Method A*—A mixture of 10.0 g. of 17a-aza-D-homo-5-androsten-3β-ol (V) (8–10) in 50 ml. of 37% formaldehyde and 50 ml. of formic acid was refluxed 5 hr. The reaction mixture was cooled, basified with ammonia solution, and extracted with ether. The ether extract was washed with water and dried, and the solvent removed *in vacuo*. Crystallization from acetonitrile gave white platelets; yield 7.5 g. (72%), m.p. 170–172° (reported 170–172°). The picrate salt crystallized as yellow needles, m.p. 264–265°, from ethanol.

Anal.—Calc. for $C_{26}H_{36}N_4O_8$: C, 58.63; H, 6.81; N, 10.52. Found: C, 58.92; H, 7.00; N, 10.66.

The methiodide of the 3-acetyl derivative of VI was prepared by treating VI with acetic anhydride and pyridine. The oily product thus obtained was treated in ethanol solution with methyl iodide. The solvent was removed *in vacuo*, and the residue was crystallized from ethanol to give white platelets, m.p. 287–289°

Anal.—Calc. for $C_{23}H_{38}INO_2$: C, 56.67; H, 7.86; I, 26.04. Found: C, 56.62; H, 7.89; I, 26.47.

Method B—A mixture of 2.0 g. of 17a-aza-D-homo-5-androsten-3β-ol (V), 30 ml. of ethanol, 10 ml. of 37% formaldehyde, and 30 mg. of platinum oxide was treated with hydrogen at 60° and 60 p.s.i. for 15 hr. The catalyst was filtered and the solvent evaporated. Crystallization of the residue from acetone yielded 1.5 g. (72%) of white platelets, m.p. 170–172°. This sample was identical to the sample prepared by Method A.

17a-Propyl-17a-aza-D-homo-5-androsten-3β-ol (VII)—17a-Aza-D-homo-5-androsten-3β-ol (V) (2.0 g.) was dissolved in a mixture of 30 ml. of ethanol and 10 ml. of propionaldehyde, and 30 mg. of platinum oxide was added. The mixture was hydrogenated at 60° and 60 p.s.i. for 15 hr. The catalyst was filtered from the mixture, and the filtrate was evaporated to dryness. The residue (1.5 g.) was chromatographed on 40 g. of neutral alumina (activity grade I). Elution with benzene–ether (1:1) gave a substance which, on crystallization from acetone, gave white needles of VII, m.p. 151–153°. The hydrobromide salt of VII was obtained as light-brown platelets, m.p. 308–309°.

Anal.—Calc. for $C_{22}H_{37}NO \cdot HBr$: C, 64.06; H, 9.28; N, 3.39; Br, 19.38. Found: C, 64.60; H, 9.31; N, 3.32; Br, 19.25.

17a-(Chloroacetyl)-17a-aza-D-homo-5-androsten-3β-ol (VIII) and 3β-(Chloroacetoxy)-17a-(chloroacetyl)-17a-aza-D-homo-5-androstene—A mixture of 2.00 g. of 17a-aza-D-homo-5-androsten-3β-ol (V), 50 ml. of methylene chloride, 100 ml. of 10% sodium hydroxide, and 10 ml. of chloroacetyl chloride was stirred 2 hr. at room temperature. The organic layer was separated, washed with water, and dried, and the solvent was removed *in vacuo*. The solid residue was crystallized from ethanol to yield 1.8 g., m.p. 170–220°. Two recrystallizations from ethanol gave 200 mg. (6%) of 3β-(chloroacetoxy)-17a-(chloroacetyl)-17a-aza-D-homo-5-androstene as white crystals, m.p. 175–177°; $\nu_{max.}^{CHCl_3}$ 1754 cm.$^{-1}$ (ester C=O) and 1656 cm.$^{-1}$ (amide C=O).

Anal.—Calc. for $C_{23}H_{33}Cl_2NO_3$: C, 62.44; H, 7.52; Cl, 16.03; N, 3.17. Found: C, 61.88; H, 7.51; Cl, 15.89; N, 3.08.

The ethanolic mother liquor was concentrated to give a substance, m.p. 240–247°. Two crystallizations from ethanol yielded 1.60 g. (64%) of VIII as white crystals, m.p. 260–261°; $\nu_{max.}^{KBr}$ 1650 cm.$^{-1}$ (amide C=O).

Anal.—Calc. for $C_{21}H_{32}ClNO_2$: C, 68.92; H, 8.81; Cl, 9.69; N, 3.38. Found: C, 68.78; H, 8.69; Cl, 9.94; N, 3.95.

17a-(3-Chloropropionyl)-17a-aza-D-homo-5-androsten-3β-ol (IX)—A solution of 2.0 g. of V in 100 ml. of methylene chloride was treated with 10 ml. of β-chloropropionyl chloride and 100 ml. of 10% sodium hydroxide solution. The mixture was stirred for 15 hr. The methylene chloride layer was separated, and the aqueous layer was extracted with methylene chloride. The combined methylene chloride extract was washed with water and dried with anhydrous sodium sulfate; the residue obtained on evaporating the solvent was 1.5 g. of IX as a white substance, m.p. 210–225°. Attempts to purify this substance further were unsuccessful. It was used as such for further reaction.

17a-(Dimethylaminoacetyl)-17a-aza-D-homo-5-androsten-3β-ol (X)—*Method C*—To a solution of 3.00 g. of VIII in 100 ml. of ethanol, 3.0 g. of dimethylamine hydrochloride and 3 g. of potassium hydroxide were added. The mixture was left at room temperature for 48 hr. It was filtered and evaporated to dryness under reduced pressure. The solid residue was washed with water, dried, and crystallized from ethanol to yield 2.7 g. (88%) of white crystals of X, m.p. 174–176°; $\nu_{max.}^{CHCl_3}$ 1645 cm.$^{-1}$ (amide C=O). The picrate of X was prepared and crystallized from ethanol to give yellow needles, m.p. 201–202°.

Anal.—Calc. for $C_{29}H_{41}N_3O_3$: C, 57.70; H, 6.85; N, 11.60. Found: C, 58.07; H, 7.37; N, 12.03.

17a-(2-Dimethylaminoethyl)-17a-aza-D-homo-5-androsten-3β-ol (XIV)—*Method D*—Compound X (2.0 g.) was dissolved in 30 ml. of dioxane, a solution of 1.0 g. of lithium aluminum hydride in 300 ml. of dioxane was added, and the mixture was refluxed for 48 hr. Excess hydride was destroyed with water. The inorganic salts were filtered and washed with dioxane. The dioxane solution was evaporated to dryness, and the residue was crystallized from ethanol to give 1.8 g. (94%) of white crystals of XIV, m.p. 195–196°. The hydrobromide salt of XIV was prepared by passing HBr gas into its acetone solution and crystallizing from ethanol to give white needles, m.p. 309–310°.

Anal.—Calc. for $C_{23}H_{40}N_2O_2 \cdot HBr$: C, 52.88; H, 8.10; N, 5.36; Br, 30.60. Found: C, 52.45; H, 7.95; N, 5.09; Br, 31.22.

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Unexpected Formation of Thioacridone and Its Spectral Properties

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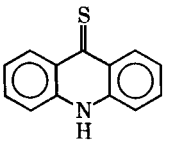
Abstract □ The isolation of thioacridone as the major product in the literature synthesis of 9-(*p*-aminophenyl)acridine and from reaction of 9-aminoacridine with *n*-butylisothiocyanate is described. Chromatographic and spectral properties of thioacridone, useful in its identification, are reported.

Keyphrases □ Thioacridone—isolation, characterization as a reaction product, spectral properties □ 9-Aminoacridine and *n*-butylisothiocyanate—isolation, characterization of thioacridone as a reaction product □ 9-(*p*-Aminophenyl)acridine with acridine, aniline, and sulfur—isolation, characterization of thioacridone as a reaction product

Current interest in these laboratories in the use of acridine derivatives as fluorescent tagging agents has led to the isolation of thioacridone [9(10*H*)-acridinethione] (I) in two separate reactions. This article describes these isolations and reports spectral data of value in the characterization of thioacridone.

Chupakhin *et al.* (1) reported the synthesis of 9-(*p*-aminophenyl)acridine in a yield of 12–13% by heating acridine, aniline, and sulfur at 185°. This yield was confirmed under similar conditions in these laboratories, but thioacridone in 83% yield also was isolated from the same reaction mixture. The formation of the thioacridone is not surprising in light of its synthesis in 85%



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yield by reaction of acridine and sulfur in a sealed tube at 190° (2) and the reported (3) high yield of 3,6-bis(dimethylamino)-9(10*H*)-acridinethione from the fusion of 3,6-bis(dimethylamino)acridine and sulfur. It is interesting that Chupakhin *et al.* (1) were able to prepare 9-(*p*-aminophenyl)-10-methylacridinium iodide from 10-methylacridinium iodide and aniline in satisfactory yield (75%), apparently without appreciable thioacridone formation. This result is in agreement with Elsager's suggestion (3) that thioacridone formation in a sulfur fusion reaction proceeds through an *N*-sulfide intermediate.

The second unexpected isolation in these laboratories of thioacridone resulted from reactions involving 9-aminoacridine and *n*-butylisothiocyanate. The result is not readily explainable based upon prior reports in the literature. Formation of thioacridone under mild conditions in this reaction, together with no indication of isothiocyanate decomposition, speaks against involvement