TABLE II[a]

ANALYTICAL DATA

Compound	M.p., °C.	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	$[\alpha]_D^{25}$b
Ia	220–221	$C_{21}H_{28}O_2$	80.73	80.84	9.03	9.23	−152°[c]
Ib	138–139	$C_{22}H_{30}O_2$	77.98	78.23	8.53	8.22	−143°[c]
Ic	129–131	$C_{23}H_{32}O_2$	77.93	77.69	8.53	8.33	−102°[c]
Id	117–118	$C_{24}H_{34}O_2$	78.22	78.09	8.75	8.60	−103°[c]
Ie	165–166	$C_{25}H_{36}O_2$	75.72	75.31	8.13	7.96	−113°[c]
If	186–188	$C_{23}H_{32}O_3$	77.61	77.10	8.29	8.27	−139°[c]
Ig	188–190	$C_{21}H_{28}O_4$	75.86	75.98	7.91	7.92	−165°[c]
IIa	147–148	$C_{21}H_{30}O_2$	79.4	79.35	9.48	8.96	−55°[c]
IIb	169–170	$C_{22}H_{32}O_2$	74.16	74.12	8.56	8.73	−83°[c]
IIc	235	$C_{26}H_{38}O_2$	79.5	79.33	10.0	9.86	−98°[c]
IId	139–149	$C_{22}H_{32}O_3$	74.80	77.18	9.40	9.55	−91°[c]
IIe	189–190	$C_{23}H_{34}O_3$	77.1	76.74	9.58	9.57	−[c]
IIIa	179–181	$C_{21}H_{26}O_3$	77.0	77.06	8.87	8.96	−71°[c]
IIIb	165–166	$C_{22}H_{28}O_3$	71.97	71.18	8.39	8.33	−91°[c]
IIIc	158–160	$C_{23}H_{30}O_3$	75.4	75.71	8.62	8.73	−92°[c]

[a] Melting points were determined on a Fisher-Johns block and are corrected. Rotations were obtained in chloroform unless otherwise noted. The analytical data were reported by Dr. R. T. Dillon and his staff at G. D. Searle & Co. [b] Pyridine. [c] Methanol.

Acylation of the 3β-Hydroxy Steroids.—A solution of 1 g. of 3β-hydroxy steroid, 5 ml. of pyridine, and 2.5 ml. of acetic or propionic anhydride was allowed to stand at 25° for 1 day. The solution was then slowly diluted at 0° with water. The crystalline precipitate which appeared was collected by filtration and dried *in vacuo*. The yields of crude product ranged between 85 and 98%. An analytical sample was prepared by crystallization of the crude product from ether and Skellysolve B or acetone and Skellysolve B.

6-Dehydro-17-ethynyltestosterone 17-Acetate.—A solution of 200 mg. of 6-dehydro-17-ethynyltestosterone, 5 ml. of pyridine, and 2 ml. of acetic anhydride was refluxed for 2 hr., cooled to 0°, diluted slowly with water, and then extracted with ether. The ether solution was washed successively with dilute hydrochloric acid, water and aqueous sodium bicarbonate and then dried over sodium sulfate and distilled to dryness *in vacuo*. The residue upon crystallization from ether and Skellysolve B yielded 150 mg. (72%) of the product which melted at 145–146°, λ_{max}^{MeOH} 282.5 mμ. (ε 26,300), $[\alpha]_D^{25}$ −86° ($CHCl_3$).

Anal. Calcd. for $C_{29}H_{28}O_3$: C, 78.37; H, 8.91. Found: C, 78.70; H, 8.17.

Synthesis of Some Steroidal [3,2-d]- and [17,16-d]-2′,6′- Diaminopyrimidines

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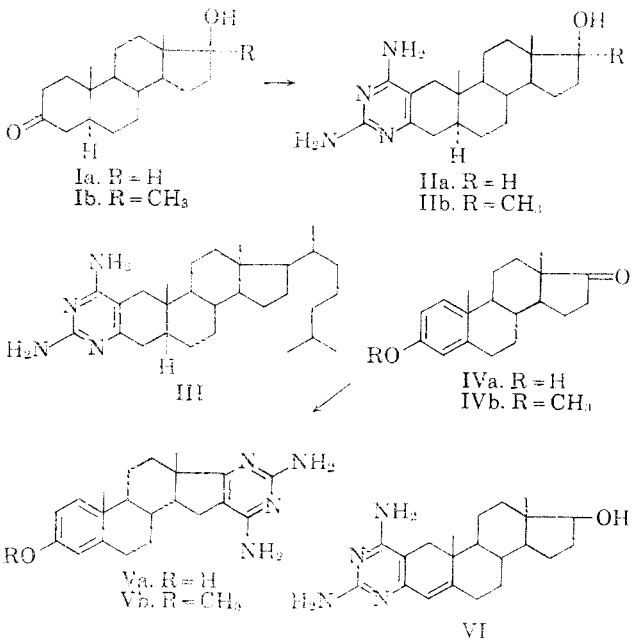
In view of recent pharmaceutical interest in steroids bearing heterocycles fused to the A- or D-ring of the steroid nucleus, we wished to synthesize steroids fused in the 2,3- and 16,17-positions to the pyrimidine ring system. The tetrahydroquinazoline synthesis of Appelquest,[1-3] employing a fusion reaction between cyanoguanidine and an appropriate cyclic ketone, formed the basis for our studies. Since the inception of this work several reports of different types of A-ring steroidal pyrimidines prepared by other methods[4-6] have appeared.

Reaction of a series of 4,5α-dihydro-3-ketosteroids with cyanoguanidine gave the anticipated steroido-[3,2-d]-2′,6′-diaminopyrimidines. Thus, 4,5α-dihydrotestosterone (Ia), 17α-methyl-4,5α-dihydrotestosterone (Ib), and 5α-cholestan-3-one gave 17β-hydroxy-5α-androstano-[3,2-d]2′,6′-diaminopyrimidine (IIa), 17β-hydroxy-17α-methyl-5α-androstano-[3,2-d]-2′,6′-diaminopyrimidine (IIb), and 5α-cholestano-[3,2-d]-2′,6′-diaminopyrimidine (IIc), respectively.

The reaction also took place with 17-ketones; thus estrone (IVa) gave 3-hydroxy-1,3,5(10)-estratrieno-[17,16-d]-2′,6′-diaminopyrimidine (Va) and estrone methyl ether (IVb) gave its respective pyrimidine Vb. Partial reaction occurred with dehydroisoandrosterone, but the product was not isolated and characterized. Reaction with testosterone gave a major product formulated as the pyrimidine VI.



The steroids II and V absorb characteristically at 283–284 mμ (ε 5000–8000) and near 230 mμ (ε 8000–16,000) in ethanol. For the dihydrotestosterone derivatives IIa and IIb the spectra were not materially changed in alkaline ethanol; however, in acidified ethanol the 230 mμ band was missing and the 284 mμ band was shifted to 273 mμ. This behavior is very similar to that of 2,4-diaminopyrimidine[7] and 2,4-diamino-5,6,7,8-tetrahydroquinazoline.[8] Other 4-aminopyrimidine derivatives behave similarly, and may lose their long wave length absorption band (present in neutral solution) on acidification, either by a substantial hypsochromic shift or by consolidation with shorter wave length absorption.[8] The diaminopyrim-

(1) A. L. Appelquest, U. S. Patent 2,517,824 (August 8, 1950).

(2) E. J. Modest, S. Chatterjee, H. Kangur, and D. M. Brun, Abstracts of Papers 137th American Chemical Society National Meeting, Cleveland, Ohio, April 5–14, 1960, p. 4N; E. J. Modest, H. Kangur, and S. Chatterjee, Abstracts of Papers, 141st American Chemical Society National Meeting, Washington, D. C., March 20–29, 1962, p. 26N; E. J. Modest, S. Chatterjee, and H. Kangur, *J. Org. Chem.*, **27**, 2708 (1962).

(3) J. DeGraw, L. Goodman, B. Wriansnia, and B. R. Baker, *ibid.*, **27**, 576 (1962).

(4) (a) J. A. Zderic, D. Halpern, H. Carara, A. Ruiz, H. C. Limon, L. Magaña, H. Jiménez, A. Bravo, and H. J. Ringold, *Chem. Ind.* (London), 1625 (1960); (b) H. J. Ringold, J. A. Zderic, C. Djerassi, and A. Bowers, U. S. Patent 3,026,317 (March 20, 1962).

(5) F. B. Colton and L. Laos, U. S. Patent 2,999,092 (Sept. 5, 1961).

(6) P. De Ruggieri, C. Gandolfi, and D. Chiaramonte, *Gazz. Chim. Ital.*, **92**, 768 (1962).

(7) M. M. Stimson, *J. Phys. Colloid Chem.*, **15**, 390 (1951).

(8) (a) R. R. Williams, A. E. Rueble, and J. Finkelstein, *J. Am. Chem. Soc.*, **59**, 526 (1937); (b) L. F. Cavalieri, A. Bendich, J. F. Tinker, and G. B. Brown, *ibid.*, **70**, 3875 (1948); (c) L. Cavalieri and A. Bendich, *ibid.*, **72**, 2587 (1950); (d) M. P. V. Boarland and J. F. W. McOmie, *J. Chem. Soc.*, 3716 (1952); (e) D. J. Brown and L. N. Short, *ibid.*, 331 (1953); (f) E. A. Falco, S. DuBreuil, and G. H. Hitchings, *J. Am. Chem. Soc.*, **73**, 3758 (1951); (g) P. B. Russell and G. H. Hitchings, *ibid.*, **73**, 3763 (1951).

idine VI derived from testosterone absorbed at 300 mμ, and this spectrum was not altered by acid or base.

The infrared spectra of the diaminopyrimidines II, III, V, and VI were dominated by three strong absorption bands in the 3 μ region, at 2.87–2.90, 2.97–3.02, and 3.14–3.17 μ, typical of diaminopyrimidines in general.[3,7,9] Further complex strong absorption occurred in the 6.15–6.69 μ region, also considered typical of diaminopyrimidines.[7,9,10]

The diaminopyrimidines IIa and IIb are weak bases with pK_a values of 7.50 and 7.45, respectively. The estrone derivatives Va and Vb, pK_a 6.70 and 6.44, respectively, are slightly stronger bases. Only one inflection could be determined in the titration curves; however, 2,4-diaminopyrimidine showed only one pK_a value (7.26).[11]

The fusion reaction was conducted in a test tube heated in an oil bath at 200–250°. Air was displaced by nitrogen. The melted steroid and cyanoguanidine were miscible in some cases, but two phases were formed with 5α-cholestan-3-one, estrone methyl ether (IVb), and dehydroisoandrosterone. In these three cases large amounts of unaltered steroid reactant were recovered and only relatively small amounts of diaminopyrimidine were formed. Generally agitation of the molten reaction mixture did not improve these cases.

The reaction product solidified on cooling and was removed, ground, and washed with water to remove melamine which was also formed in the reaction. The steroidal products possessed at this stage the characteristic ultraviolet and infrared absorption properties of the purified materials, and despite constant spectral properties satisfactory elemental analyses could not be obtained in certain cases. The preparations were readily solvated and were electrostatic. Chromatographic purity of each preparation was attained, as evidenced by both thin-layer and paper chromatographic procedures.

The two dihydrotestosterone derivatives IIa and IIb had a low order of androgenic activity with no anabolic activity.[12] The estrone derivative Va had low order (0.1% of estrone) estrogenic activity, but neither Va nor Vb exhibited antilipemic effects.[13]

In view of the variety of antimicrobial activities of other 2,4-diaminopyrimidines, including folic acid-folinic acid antagonism,[14] antimalarial activity,[8f,8g,14a,15] anticoccidial activity,[16] antileukemic and antitumor activity,[17] antibacterial activity,[18] etc., the diaminopyrimidines IIa, IIb, Va, and Vb were tested against a variety of bacteria and fungi. Antibacterial activity was found for all four against several Gram-positive organisms, and particularly against *Staphylococcus aureus* strains (Table I).

TABLE I

ANTIBACTERIAL ACTIVITIES OF STEROID [3,2-d]- AND [17,16-d]-2′,6′-DIAMINOPYRIMIDINES

	Minimum inhibitory concentration, μg./ml. (Agar serial dilution)				
Test organism	IIa	IIb	Va	Vb	Potassium Benzyl Penicillin
Staphylococcus aureus 209P	31.3	100	10	10	0.05
Staphylococcus aureus 53-180	250	100	10	10	>100
Staphylococcus aureus CHP	250	..	10	10	100
Staphylococcus aureus Smith	31.3	..	..	..	..
Staphylococcus aureus J 144	..	..	..	10	..
Streptococcus pyogenes Group A	31.3	..	..	..	..
Diplococcus pneumoniae 37	31.3	..	..	..	..
Sarcina lutea	31.3	..	..	..	..
Gaffkya tetragena	31.3	..	..	..	..
Salmonella paratyphi	250	100	50	10	0.10
Brucella bronchiseptica	500	100	50	25	5.0
Neisseria catarrhalis	250	500	50	25	..
Lactobacillus casei	50	50	25	25	..
Bacillus subtilis 6633	25	50	25	10	0.025
Bacillus subtilis SR	250	1000	250	25	>100
Mycobacterium sp.	25	..	>1000	1000	>100
Pseudomonas aeruginosa	1000	>1000	>1000	1000	>100
Escherichia coli 6880	1000	1000	500	1000	10
Escherichia coli SR	1000	1000	500	1000	50

However, neither IIa nor Va protected mice challenged intraperitoneally with a penicillin resistant strain of *S. aureus* CHP.

Experimental[19]

17β-Hydroxy-5α-androstano-[3,2-d]-2′,6′-diaminopyrimidine (IIa).—4,5α-Dihydrotestosterone (1 g.) was mixed with 400 mg. of cyanoguanidine and placed in an oil bath preheated to 230°. The material melted in a few minutes and was heated at 230–250° for 30 min., during which time bubbles evolved. The cooled mass was powdered, washed with water, and dried, yielding 1.275 g., λ_{max} 284 mμ (ε 5000). The solids were dissolved in ethanol, precipitated with water, and the product dried over phosphorus pentoxide, yielding 990 mg. of material with λ_{max} 284 mμ (ε 5160), which was redissolved in tetrahydrofuran and reprecipitated with water. The white solids were extracted with boiling benzene, and the filtered extracts evaporated under vacuum. The pure product was dried thoroughly over phosphrous pentoxide under vacuum, m.p. 190°, and at 272–278° dec. (with shrinking and coloration from 243°); λ_{max} 284 mμ (ε 4830),

(9) L. N. Short and H. W. Thompson, *J. Chem. Soc.*, 168 (1952).

(10) The two regions of complex absorption have also been reported for some monoaminopyrimidines bearing the fused steroidal nucleus (see references 5 and 6).

(11) A. Albert, R. Goldacre, and J. Phillips, *J. Chem. Soc.*, 2240 (1948).

(12) Using the test of L. G. Hershberg, E. G. Shipley, and R. K. Meyer, *Proc. Soc. Exptl. Biol. Med.*, **83**, 175 (1953), IIa was 3–10%, IIb was 0.6% as potent as testosterone propionate (ventral prostate). Potency of IIa and IIb (*levator ani*) was less than 1.5% compared with testosterone propionate.

(13) Estrogenic activity was measured by mouse uterine growth, *cf.* B. L. Rubin, A. Dorfman, L. Black, and R. I. Dorfman, *Endocrinology*, **49**, 429 (1951). The antilipemia test employed was one devised by Dr. R. A. Edgren using intact adult male rats on a normal diet, with test compound administered over 9 days. Active compounds reduce the Liebermann-Burchard positive lipids of whole plasma extracts. Testicular atrophy was also noted as a sign of "feminization."

(14) (a) G. H. Hitchings, G. B. Elion, H. VanderWerff, and E. A. Falco, *J. Biol. Chem.*, **174**, 765 (1948); (b) E. A. Falco, G. H. Hitchings, P. B. Russell, and H. VanderWerff, *Nature*, **164**, 107 (1949); (c) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell, M. B. Sherwood, and H. VanderWerff, *J. Biol. Chem.*, **183**, 1 (1950); (d) G. H. Hitchings, *Trans. Roy. Soc. Trop. Med. Hyg.*, **46**, 467 (1952); (e) G. H. Hitchings, E. A. Falco, G. B. Elion, S. Singer, G. B. Waring, D. J. Hutchison, and J. H. Burchenal, *Arch. Biochem. Biophys.*, **40**, 479 (1952); (f) G. H. Hitchings, *Congr. intern. biochim., Résumés communs., 2e Congr.*, Paris, **1952**, p. 83; (g) E. J. Modest, G. E. Foley, and S. Farber, *Acta Unio Intern. Contra Cancrum*, **16**, 702 (1960).

(15) (a) L. G. Goodwin, *Nature*, **164**, 1133 (1949); (b) E. A. Falco, P. B. Russell, and G. H. Hitchings, *J. Am. Chem. Soc.*, **73**, 3753 (1951); (c) E. A. Falco, L. G. Goodwin, G. H. Hitchings, I. M. Rollo, and P. B. Russell, *Brit. J. Pharm. Chemother.*, **6**, 185 (1951); (d) I. M. Rollo, *Trans. Roy. Soc. Trop. Med. Hyg.*, **46**, 474 (1952); (e) L. G. Goodwin, *ibid.*, **46**, 485 (1952); (f) G. R. Coatney, *ibid.*, **46**, 496 (1952).

(16) R. E. Lux, *Antibiotics and Chemotherapy*, **4**, 971 (1954).

(17) (a) J. H. Burchenal, S. K. Goetchius, C. C. Stock, and G. H. Hitchings, *Cancer Res.* **12**, 251 (1952); (b) D. A. Clarke, S. M. Buckley, S. S. Sternberg, C. C. Stock, C. P. Rhoads, and G. H. Hitchings, *ibid.*, **12**, 255 (1952); (c) K. Sugiura, *ibid.*, **13**, 431 (1953).

(18) E. F. Rogers, W. J. Leanza, and L. H. Sarett *J. Org. Chem.*, **22**, 1492 (1957).

(19) Melting points were taken on a Kofler block under a microscope and are corrected. Infrared spectra were determined on pressed potassium bromide disks using the Perkin-Elmer Model 21 instrument. Ultraviolet absorption spectra were determined on solutions in 95% ethanol. The p*K* values were determined on 50% aqueous ethanol solutions using 0.1 *N* HCl.

230 mμ (ε 8340); λ_{min} 256 mμ (ε 1520), 224 mμ (ε 8240); $\lambda_{max}^{EtOH-HCl}$ 273 mμ (ε 4520), λ_{min} 256 mμ (ε 3740); λ_{max}^{KBr} 2.90 (shoulder), 3.01, 3.17, 6.21, 6.32, 6.38, 6.95, 9.50, 9.75, 12.20 μ, etc.

Anal. Calcd. for $C_{21}H_{32}N_4O$: C, 70.75; H, 9.05; N, 15.72. Found: C, 70.20; H, 9.07; N, 13.41.

17β-Hydroxy-17α-methyl-5α-androstano-[3,2-d]-2′,6′-diaminopyrimidine (IIb).—Two grams of 17α-methyl-4,5α-dihydrotestosterone and 1.0 g. of cyanoguanidine were mixed in a test tube and placed in an oil bath preheated to 175°. Heating was continued, the materials melting at 187–200°. At 220° bubbles evolved from the reaction melt, and heating was then continued for 30 min. The cooled melt (2.61 g.) was ground and extracted with benzene in a Soxhlet extractor for several days. Six separate extracts were taken, which yielded on evaporation the purified pyrimidine, 1.885 g., m.p. 247–250° dec. (shrinking and coloration from 172°); λ_{max} 284 mμ (ε 5000–5300). After dissolving in ethanol, precipitation with water, drying, reextraction into benzene, evaporation, and thorough drying over phosphorus pentoxide the product melted at 170–175° and at 248–256° dec.; $[\alpha]_D$ +16° (1%, EtOH); λ_{max} 284 mμ (ε 5440), 230 mμ (λ9650, shoulder); λ_{min} 254 mμ (ε 1970); $\lambda_{max}^{EtOH-HCl}$ 273 mμ (ε 5330), λ_{min} 257 mμ (ε 4280); λ_{max}^{KBr} 2.87 (shoulder), 3.00, 3.17, 3.45, 3.52 (shoulder), 6.19, 6.29, 6.39, 6.95, 9.19, 10.72, 12.69 μ, etc.

Anal. Calcd. for $C_{22}H_{34}N_4O$: C, 71.31; H, 9.24; N, 15.12. Found: C, 71.67; H, 8.57; N, 13.57.

Solvated forms were also obtained, a hydrate, m.p. 179–182° and 252–260° dec.

Anal. Calcd. for $C_{22}H_{34}N_4O \cdot 1^1/_4H_2O$: C, 67.23; H, 9.36; N, 14.26. Found: C, 67.08; H, 8.70; N, 14.63.

5α-Cholestano-[3,2-d]-2′,6′-diaminopyrimidine (III).—A mixture of 1.0 g. of 5α-cholestan-3-one and 0.25 g. of cyanoguanidine was heated at 250° under nitrogen with vigorous stirring. After melting, two phases were formed. After 15 min. an additional 0.25 g. of cyanoguanidine was added and heating was continued for 15 min. The cooled reaction mixture (1.0 g.) was washed with 100 ml. of hot water twice, yielding 0.91 g. of yellow solids, m.p. 200–208° (softening from 100°); $\lambda_{max}^{dimethylsulfoxide}$ 298 mμ (ε 6730); λ_{max}^{KBr} 3.05, 3.20, 5.85 (residual 5α-cholestanone), 6.17, 6.39, 6.97 μ, etc. Thin-layer chromatography (5% ethyl acetate in hexane) using 25% antimony trichloride in chloroform for detection indicated the single product (R_f 0.48) together with unaltered 5α-chloestanone. No further purification was attempted on this preparation.

3-Hydroxy-1,3,5(10)-estratrieno-[17,16-d]-2′,6′-diaminopyrimidine (Va).—Estrone (6 g.) and 4.0 g. of cyanoguanidine were well mixed in a test tube, from which air was excluded by a stream of nitrogen. The mixture was placed in a preheated oil bath (260°). Within 3–5 min. the reactants melted. The molten mass was stirred under nitrogen at 260° until bubbling ceased and the mass became viscous (5–7 min.). The cooled, powdered mass was slurry-washed with (70°) water twice, then dissolved in 400 ml. of warm (70°) water acidified to pH 1 with concentrated hydrochloric acid. After filtration of insolubles the filtrate was cooled and neutralized with concentrated ammonium hydroxide, and the precipitated product filtered. Solution and precipitation were repeated 3 more times, yielding a crude product weighing 3.9 g. The pyrimidine was extracted with boiling ethyl acetate, and precipitated with petroleum ether, affording 536 mg. of purified pyrimidine, m.p. 320–330° dec.; $[\alpha]_D$ +100° (1%, MeOH); λ_{max} 283 mμ (ε 8870), 230 mμ (ε 15600, shoulder), λ_{min} 257 mμ (ε 2180); λ_{max}^{KBr} 2.87, 2.97, 6.26, 6.69, 6.85, 7.02 μ, etc.

Anal. Calcd. for $C_{20}H_{24}N_4O \cdot {}^1/_3H_2O$: C, 70.19; H, 7.25; N, 16.37. Found: C, 70.11; H, 7.24; N, 16.50.

3-Methoxy-1,3,5(10)-estratrieno-[17,16-d]-2′,6′-diaminopyrimidine (Vb).—A solution of 1.00 g. of 3-hydroxy-1,3,5(10)-estratrieno-[17,16-d]-2′,6′-diaminopyrimidine, 10 g. of potassium hydroxide, 25 ml. of water, and 25 ml. of methanol was held at 35° with stirring while 10 ml. of dimethyl sulfate was added over 10 min. Stirring was continued for 2 hr., at which time 50 ml. of water was added. After stirring for an additional 15 min. the product (417 mg.) was filtered. Recrystallization from boiling methanol yielded 316 mg. of product, with a second crop of 45 mg. The pure product melted 232–239° dec.; $[\alpha]_D$ +92° (1%, EtOH); λ_{max} 283 mμ (ε 8160), 230 mμ (ε 16,300, shoulder), λ_{min} 257 mμ (ε 2100); λ_{max}^{KBr} 2.92, 3.02, 3.14, 6.15, 6.33, 6.93 μ, etc.

Anal. Calcd. for $C_{21}H_{26}N_4O \cdot H_2O$: C, 68.10; H, 7.68; N, 15.13. Found: C, 68.08; H, 7.61; N, 14.63.

Fusion of 1.0 g. of estrone methyl ether with 330 mg. of cyanoguanidine at 240° gave two layers of melted reactants. After cooling the solids were extracted with hot water, and the dried residue was extracted with acidified water (pH 1). On neutralization of the acid extract 95 mg. of Vb was precipitated, identified by thin-layer chromatography and infrared spectra. The acid insoluble residue weighed 831 mg. and was identified as unaltered estrone methyl ether.

17β-Hydroxy-4-androsteno-[3,2-d]-2′,6′-diaminopyrimidine (VI).—A mixture of 5 g. of testosterone and 2 g. of cyanoguanidine was heated at 250° for 30 min. under nitrogen with vigorous stirring. The mixture melted after about 3 min., but solidified after about 20 min. of heating. After cooling the red-brown solids were extracted twice with 500 ml. portions of hot water, leaving 5.0 g. of residue, which was then extracted twice with 500 ml. portions of boiling benzene. On cooling, the benzene extracts deposited 1.55 g. of product. Of this material, 200 mg. was dissolved in hot water acidified to pH 1 with hydrochloric acid and filtered hot. The filtrate was cooled to 0° and adjusted to pH 9 with ammonium hydroxide, precipitating 110 mg. of yellow solids, m.p. 218–227°; λ_{max} 238 mμ (ε 14,600), 260 mμ (ε 9550, shoulder), and 300 mμ (ε 5020); λ_{max}^{KBr} 2.87, 3.02, 3.15, 6.15, 6.40, 6.95, 9.49 μ, etc.

Anal. Calcd. for $C_{21}H_{36}N_4O \cdot H_2O$: C, 67.71; H, 8.66; N, 15.04. Found: C, 68.55; H, 8.63; N, 14.81.

The product was free from cyanoguanidine and melamine as evidenced by paper chromatography using Turnbull blue and *o*-tolidine reagents. Thin-layer chromatography indicated a trace of testosterone remaining.

Evaporation of the initial water extracts yielded a solid residue, identified as melamine by its infrared spectra.

Chromatography.—The homogeneity of each pyrimidine was examined by thin-layer chromatography using silica gel chromatoplates bound with rice starch.[20] Freedom from steroidal ketone starting material was established using a solvent system of benzene–ethanol (3:2); detection of both steroidal ketone and pyrimidine product was accomplished with a 10% solution of phosphomolybdic acid in ethanol. Paper chromatography on Whatman no. 1 paper with the solvent system benzene–acetone–water (2:1:2) was used to establish freedom from melamine and cyanoguanidine.

Both melamine, cyanoguanidine, and the diaminopyrimidines were detected with Turnbull's blue reagent[21] and with a modified *o*-tolidine reagent of Habermann.[22] The dried paper chromatogram was dipped in a mixture of equal volumes of ethanol and acetone, blotted, and exposed to an atmosphere of chlorine (generated from 30 ml. of concentrated hydrochloric acid, 20 ml. of water, 100 ml. of 1% potassium permanganate solution) in a glass jar for 10 min. The paper was then immersed in a freshly prepared solution of equal parts of 0.05 *M* potassium iodide solution and of a saturated solution of *o*-tolidine in 2% acetic acid. Melamine (R_f 0.0) and cyanoguanidine (R_f 0.02) appear immediately as intense blue spots. The steroidal pyrimidines (except for III and VI) appear more slowly as blue spots with much less color intensity, with mobility behavior: IIa, R_f 0.05; IIb, 0.1 cm./h.; Va, R_f 0.33; Vb, R_f 0.77; VI, R_f 0.19.

The nitrogenous steroids and cyanoguanidine and melamine also responded to the *o*-tolidine procedure of Barrollier,[23] where the transient blue spots become permanently purple on treatment with 0.1% ammonium molybdate in *N* acetic acid.

Acknowledgment.—The authors are indebted to Drs. G. Warren, S. Rosenman, and J. Yurchenko for the microbiological assays, to Dr. R. Edgren for the endocrinological assays, and to Dr. T. S. Osdene for calling to our attention the cyanoguanidine fusion synthesis of pyrimidines.

(20) L. L. Smith and T. Foell, *J. Chromatography*, **9**, 339 (1962).

(21) G. M. Barron, R. S. Evans, and J. A. F. Gardner, *Nature*, **170**, 249 (1952).

(22) V. Habermann, *Biokhimiya*, **25**, 891 (1960); *Biochemistry* (USSR) (English translation), **25**, 689 (1960).

(23) J. Barrollier, *Naturwissenschaften*, **48**, 554 (1961).