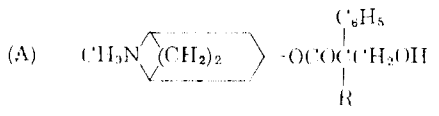
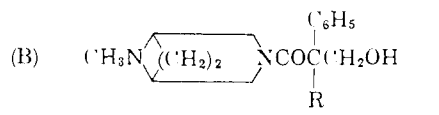
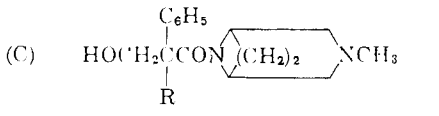
TABLE I

(A) 

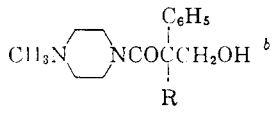
Compd.	Isomer	R	Ref.	*In vitro* activity,[a] (γ/ml.)	Relative potency
I	± Atropine			0.016	100
II		CH_2OH	3	.025	64
III	(±)	CH_3	1	.018	89
IIIa	(−)	CH_3	1	.008	200
IIIb	(+)	CH_3	1	.300	5

(B) 

IV	(±)	H	4	0.180	9
V	(±)	CH_3	4	.040	40
Va	(−)	CH_3	4	.020	80
Vb	(+)	CH_3	4	>10	0

(C) 

VI	(±)	CH_3	5	0.350	5

(D) 

VII[c]	(±)	H	...	0.190	8
VIII[d]	(±)	CH_3	...	.600	3
VIIIa[e]	(−)	CH_3	...	.400	4

[a] Concentration able to reduce to 50% the contractile response of isolated intestine to acetylcholine. [b] These compounds were prepared starting from N-methylpiperazine by a procedure similar to that described[4,5] for IV–VI. [c] Yield, 48%; m.p. 102–103° (Et_2O). *Anal.* Calcd. for $C_{14}H_{20}N_2O_2$: C, 67.74; H, 8.12; N, 11.28. Found: C, 67.92; H, 8.27; N, 10.92. [d] Yield, 68%; m.p. 225–228° (HCl salt) (EtOH). *Anal.* Calcd. for $C_{15}H_{23}ClN_2O_2$: N, 9.37; Cl, 11.86. Found: N, 9.02; Cl, 12.06. [e] Yield, 55%; m.p. 213–215° (HCl salt) (EtOH). *Anal.* Calcd. for $C_{15}H_{23}ClN_2O_2$: N, 9.37; Cl, 11.86. Found: N, 9.51; Cl, 11.91. $[\alpha]D^{20}$ −45.4° (*c* 1, H_2O).

by N-methylpiperazine or by 3-methyl-3,8-diazabicyclo[3.2.1]octane, a decrease in activity was observed.

As far as a comparison of the anticholinergic activity in the tropoyl and α-methyltropoyl series is concerned, α-methyltropoyl amides are more active than the corresponding tropoyl derivative in the 8-methyl-3,8-diazabicyclo[3.2.1]octane series (B), but are less active in the N-methylpiperazine series (D).

The weak anticholinergic activity of (±)-3-methyl-8-α-methyltropoyl-3,8-diazabicyclo[3.2.1]octane (VI) may be correlated with the lack of structural similarity with the active isomer V. This result offers an indirect support for the assumed[4] pharmacological analogy of 8-methyl-3,8-diazabicyclo[3.2.1]octane (parent compound of V) and tropane.

Acknowledgment.—The authors are grateful to Dr. A. Wittgens for her assistance in revising the manuscript.

Steroidal Heterocycles. X.[1] Steroidal[3,2-*d*]pyrimidines and Related Compounds

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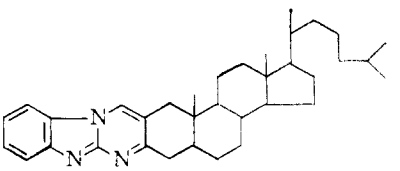
As part of our continuing program on the synthesis of steroidal heterocycles, we have prepared a series of steroidal[3,2-*d*]pyrimidines. Prior to our initial report in the field of steroidal heterocycles,[2] no steroidal pyrimidines had been reported.[3] Subsequently, several other groups have reported on steroidal[3,2-*d*]-pyrimidines.[4]



Our method for preparing the steroidal[3,2-*d*]pyrimidines employed the same intermediates, the 2-hydroxymethylenesteroids, as used previously with other steroidal heterocycles.[2,5,6] These intermediates were converted to the corresponding steroidal pyrimidines by the procedure of Baumgarten, *et al.*,[7] or a modification of this method. With this procedure, the pyrimidine ring was formed from the 2-hydroxymethylenesteroids and an amidine hydrochloride. Triethylamine was generally the catalyst employed and ethanol was usually the solvent. Baumgarten, *et al.*,[7] reported yields of 23–43% in their preparation of nonsteroidal pyrimidines. Our yields generally ran lower, but with the aid of chromatography, the steroidal pyrimidines could be isolated readily. 17β-Hydroxy-2-hydroxymethylene-5α-androstan-3-one (**1**) and acetamidine hydrochloride gave 17β-hydroxy-5α-androstano[3,2-*d*]-2′-methylpyrimidine (**2**). A side product was isolated from the acid-soluble part of the reaction mixture and shown to be 2-aminomethylene-17β-hydroxy-5α-androstan-3-one (**3**).

Similarly, when 17β-hydroxy-2-hydroxymethylene-17β-methyl-5α-androstan-3-one (**4**) was treated with acetamidine and formamidine hydrochlorides, the pyrimidines **5** and **6**, respectively, were obtained. The enamine **7** was isolated from the reaction mixture from

(1) Paper IX: P. E. Shaw, F. W. Gubitz, K. F. Jennings, and R. L. Clarke, in press.

(2) R. O. Clinton, A. J. Manson, F. W. Stonner, A. L. Beyler, G. O. Potts, and A. Arnold, *J. Am. Chem. Soc.*, **81**, 1513 (1959).

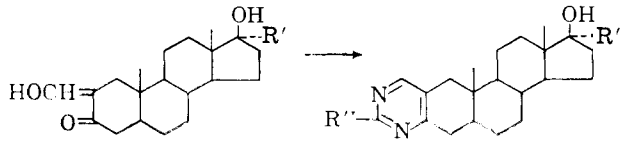
(3) H. Antaki and V. Petrow, *J. Chem. Soc.*, 901 (1951), have reported 1′,9′,11′-triazafluoreno(3′,2′:2,3)cholestane.

(4) (a) T. Colton and I. Loas, U. S. Patent 2,999,092 (September 5, 1961); (b) J. Zderic, B. Halpern, H. Carpio, A. Ruiz, D. Limon, L. Magaña, H. Jiménez, A. Bowers, and H. Ringold, *Chem. Ind.* (London), 1625 (1960); (c) P. Ruggeri, C. Gandolfi, and D. Chiaramonti, *Gazz. Chim. Ital.*, **92**, 768 (1962).

(5) A. J. Manson, F. W. Stonner, H. C. Neumann, R. G. Christiansen, R. L. Clarke, J. H. Ackerman, D. F. Page, J. W. Dean, D. K. Phillips, G. O. Potts, A. Arnold, A. L. Beyler, and R. O. Clinton, *J. Med. Chem.*, **6**, 1 (1963).

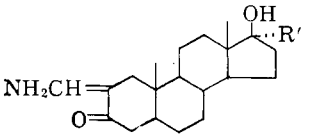
(6) R. O. Clinton, A. J. Manson, F. W. Stonner, H. C. Neumann, R. G. Christiansen, R. L. Clarke, J. H. Ackerman, D. F. Page, J. W. Dean, W. B. Dickinson, and C. Carabateas, *J. Am. Chem. Soc.*, **83**, 1478 (1961).

(7) H. E. Baumgarten, P. L. Creger, and C. E. Villars, *ibid.*, **80**, 6609 (1958).



1 (R′ = H)
4 (R′ = CH_3)

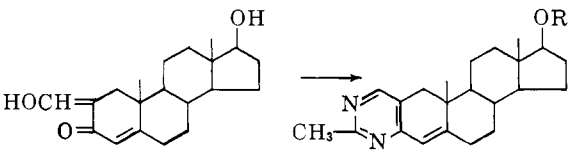
2 (R′ = H; R″ = CH_3)
5 (R′ = R″ = CH_3)
6 (R′ = CH_3, R″ = H)



3 (R′ = H)
7 (R′ = CH_3)

the preparation of the pyrimidine **5** and also appeared to be formed in the preparation of **6.**

In the preparation of the pyrimidine 8 from 2-hydroxymethylenetestosterone (**9**),[8] it was found advantageous to use dimethylformamide as the solvent or sodium ethoxide as the catalyst. The pyrimidine 8 did not crystallize, but could be acetylated to give the corresponding crystalline 17β-acetoxypyrimidine (**10**).



9

8 (R = H)

10 (R = $CH_3C(=O)$—)

The myotrophic and androgenic activities of these steroids were determined by a modification of the method of Hershberger, Shipley, and Meyer.[9] Immature male rats of the Sprague-Dawley strain, 22 days of age (41–44 g.) were castrated and maintained on laboratory chow fed *ad libitum*. Each compound was administered subcutaneously or orally daily except Sunday for 9 days, starting 7 days after castration. The animals were autopsied on the 17th post-castration day, 24 hr. after the last medication. The levator ani muscle and ventral prostate were excised, blotted, and weighed on a microtorsion balance. 17β-Hydroxy-17α-methyl-5α-androstano[3,2-*d*]-2′-methylpyrimidine (**5**) was of the order of $^1/_{16}$ as myotrophic and $^1/_{64}$ as androgenic as testosterone propionate when administered subcutaneously. Orally, **5** was as myotrophic and 0.33 as androgenic as methyl testosterone. 17β-Hydroxy-5α-androstano[3,2-*d*]-2′-methylpyrimidine (**2**) was comparable in activities to the corresponding 17-methyl analog when administered parenterally. The myotrophic and androgenic activities of 17β-acetoxyandrost-4-eno[3,2-*d*]-2′-methylpyrimidine (**10**) were likewise of the same order as those of **5**. It should be noted that the desmethyl analog, 17β-hydroxy-17α-methyl-5α-androstano[3,2-*d*]pyrimidine (**6**), was reported to be inactive.[4c]

(8) F. Weisenborn, D. Remy, and T. Jacobs, *J. Am. Chem. Soc.*, **76**, 552 (1954).

(9) L. G. Hershberger, E. G. Shipley, and R. K. Meyer, *Proc. Soc. Exptl. Biol. Med.*, **83**, 175 (1953).

Experimental[10]

17β-Hydroxy-5α-androstano[3,2-*d*]-2′-methylpyrimidine (2).—A mixture of 6.40 g. (0.020 mole) of 17β-hydroxy-2-hydroxymethylene-5α-androstan-3-one[6] (**1**), 2.3 g. (0.024 mole) of acetamidine hydrochloride, 3.45 ml. (0.025 mole) of triethylamine, and 16 ml. of anhydrous ethanol was refluxed for 27 hr. The cooled reaction product was added to 160 ml. of 2.5 *N* hydrochloric acid and this mixture was extracted 3 times with ether. The combined ether extracts were washed with a little dilute hydrochloric acid and discarded. The combined aqueous layers were made strongly basic with sodium hydroxide solution. The basic mixture was extracted 3 times with ether and the combined ether extracts were dried over sodium sulfate. The ethereal mixture was filtered and the ether was removed on a steam bath. There was obtained 4.1 g. of yellow oil after drying the residue under vacuum. When this oil was heated with ethyl acetate–benzene, some crystalline material separated. The mixture was cooled and the solid was removed by filtration and dried. There was obtained 0.67 g. of yellow solid, m.p. 180–230°. Several recrystallizations of this solid from ethanol–benzene gave yellow crystals, m.p. 256–259°, of **2-aminomethylene-17β-hydroxy-5α-androstan-3-one (3),** λ_{max} 315 mμ (ε 15,300); λ_{max} 3.07, 3.45, 6.09, 6.73, 6.88 μ.

Anal. Calcd. for $C_{20}H_{31}NO_2$: C, 75.67; H, 9.84; N, 4.41. Found: C, 75.91; H, 9.94; N, 4.17.

The mother liquors from the above enamine were evaporated to dryness. The residue was dissolved in benzene and added to a column of 220 g. of Florisil[11] prewet with benzene. The column was eluted with benzene and gradually with increasing amounts of ether in benzene. When the column was eluted with 15–20% ether in benzene, a total of 2.0 g. (29%) of crystalline material was obtained. Three recrystallizations of this material from ethyl acetate–hexane gave colorless clusters of needles, m.p. 163.4–165.2°; [α]D +56.2°; λ_{max} 260 mμ (ε 4500); λ_{max} 2.96, 3.43, 6.32, 6.41, 6.92 μ.

Anal. Calcd. for $C_{22}H_{32}N_2O$: C, 77.60; H, 9.47; N, 8.23. Found: C, 77.76; H, 9.19; N, 7.87.

17β-Hydroxy-17α-methyl-5α-androstano[3,2-*d*]-2′-methylpyrimidine (5).—A mixture of 6.65 g. (0.020 mole) of 17β-hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one[6] (**4**), 2.3 g. (0.024 mole) of acetamidine hydrochloride, 3.45 ml. (0.025 mole) of triethylamine, and 16 ml. of anhydrous ethanol was refluxed for 24 hr. The product was isolated in the same manner described above for **2.** The residue was a mixture of solid and orange oil weighing 4.5 g. This residue was treated with a little ether and the insoluble solid was removed by filtration. There was obtained 0.92 g. of yellow solid, m.p. 266–270°. Recrystallization of this material from ethanol gave yellow prisms, m.p. 274–276°, of **2-aminomethylene-17β-hydroxy-17α-methyl-5α-androstan-3-one** (7); λ_{max} 315 mμ (ε 14,600); λ_{max} 3.08, 3.45, 6.10, 6.24, 6.77 μ.

Anal. Calcd. for $C_{21}H_{33}O_2N$: C, 76.09; H, 10.03; N, 4.23. Found: C, 75.91; H, 10.00; N, 4.12.

After removal of the above solid, the remainder of the crude product was combined with 1.1 g. of crude product from a similar half-scale reaction. Following evaporation of the solvent, the residue was dissolved in benzene and added to a column of 250 g. of Florisil[11] prewet with benzene. The column was eluted with benzene and gradually increasing amounts of ether in benzene. When the column was eluted with 15–20% ether in benzene, 2.86 g. (27%) of crystalline material was obtained. Two recrystallizations of the material from ethyl acetate–hexane gave 0.9 g. (8%) of almost colorless clusters of plates, m.p. 161.2–163.4°, [α]D +31.7°; λ_{max} 260 mμ (ε 4500); λ_{max} 2.94, 3.43, 6.32, 6.42, 6.92 μ.

Anal. Calcd. for $C_{23}H_{34}N_2O$: C, 77.92; H, 9.67; N, 7.90. Found: C, 77.63; H, 9.64; N, 7.82.

17β-Hydroxy-17α-methyl-5α-androstano[3,2-*d*]pyrimidine (6).—A mixture of 6.60 g. (0.020 mole) of 17β-hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one[6] (**4**), 2.3 g. (*ca.* 0.024 mole) of crude formamidine,[12] 3.4 ml. (0.025 mole) of triethylamine, and 15 ml. of anhydrous ethanol was refluxed for

(10) Melting points were taken in a Hershberg-type apparatus and are corrected. Rotations were taken in chloroform solution at 25°, *c* ~ 1%; ultraviolet spectra in 95% ethanol (Cary) and infrared spectra in a potassium bromide disk (Perkin-Elmer 21).

(11) The Floridin Company, Tallahassee, Fla.

(12) D. Brown, *J. Appl. Chem.*, **2**, 202 (1952).

20 hr. The product was isolated in the manner described for 2. During the final ether extractions, some ether-insoluble solid separated and was removed by filtration. The dried weight was 2.3 g., m.p. 230–240°; λ_{max} 315 mμ (ε 11,100). This solid is undoubtedly the enamine 7 but was not further characterized. Evaporation of the ether extracts gave 1.7 g. of solid, m.p. 170–178°. This residue was combined with 0.55 g. of crude product from a similar half-scale reaction. The combined crude products were dissolved in benzene and added to a column of 190 g. of Florisil[11] prewet with benzene. The column was eluted with benzene and increasing amounts of ether in benzene. When the column was eluted with 15–20% ether in benzene, 0.65 g. (6%) of crystalline material was obtained. Two recrystallizations of this material from benzene gave 0.43 g. (4%) of colorless prisms, m.p. 205.6–207.2°, $[\alpha]_D$ +29.7°; λ_{max} 254 mμ (ε 4100); λ_{max} 2.99, 3.45, 6.33, 6.38, 6.82, 7.00, 7.12 μ.

Anal. Calcd. for $C_{32}H_{32}N_2O$: C, 77.60; H, 9.47; N, 8.23. Found: C, 77.43; H, 9.31; N, 8.01.[13]

17β-Acetoxyandrost-4-eno[3,2-*d*]-2′-methylpyrimidine (10). A.—A solution of sodium ethoxide in ethanol was prepared from 0.69 g. (0.030 mole) of sodium metal and 20 ml. of anhydrous ethanol and added to a mixture of 6.32 g. (0.020 mole) of 2-hydroxymethylenetestosterone[8] (**9**), 2.82 g. (0.030 mole) of acetamidine hydrochloride, and 50 ml. of anhydrous ethanol. The mixture was allowed to stand for 0.5 hr. at room temperature and then refluxed for 6 hr. The crude product was isolated as usual to give 1.25 g. of an amber oil. This oil was combined with 0.3 g. of oil from a similar half-scale reaction. The combined crude products were dissolved in benzene and added to a column of 80 g. of Florisil[11] prewet with benzene. The column was eluted with benzene and gradually increasing amounts of ether in benzene. When the column was eluted with 20% ether in benzene, 0.76 g. (7%) of oil was obtained, λ_{max} 227 mμ (ε 10,600), 250 mμ (5500), 301 mμ (10,400).

A mixture of 0.69 g. of this oil, 4 ml. of pyridine, and 4 ml. of acetic anhydride was heated for 10 min. on a steam bath and then poured into water. The mixture stood for several hr. and the solid was removed by filtration. There was obtained 0.59 g. (6%) of colorless solid, m.p. 150–165°. Repeated recrystallizations from hexane gave colorless prisms, m.p. 170.4–171.8°; $[\alpha]_D$ +143.4°; λ_{max} mμ (11,700), 256 mμ (5300), 302 mμ (12,600); λ_{max} 3.45, 5.76, 6.14, 6.37, 6.46, 6.96 μ.

Anal. Calcd. for $C_{34}H_{32}N_2O_2$: C, 75.75; H, 8.48; N, 7.36. Found: C, 75.60; H, 8.51; N, 7.43.

B.—A mixture of 3.16 g. (0.0100 mole) of 2-hydroxymethylenetestosterone,[8] 1.16 g. (0.012 mole) of acetamidine hydrochloride, 1.73 ml. (0.0125 mole) of triethylamine, and 10 ml. of dimethylformamide was refluxed for 2 hr. The solution was poured into 330 ml. of *N* hydrochloric acid and the crude product isolated in the manner described above. There was obtained 0.96 g. of an amber oil. This oil was converted to the acetate **10** by the above procedure and the crude oily product was chromatographed on Florisil.[11] After preliminary elution with benzene, a mixture of 15% ether in benzene removed the crystalline acetate **10** identical with the product previously described.

Acknowledgment.—The authors are indebted to Dr. F. C. Nachod, Miss Catherine Martini, and Mr. M. Priznar for spectral determinations, to Mr. K. D. Fleischer and staff for analytical services, and to Drs. F. W. Stonner and A. J. Manson for helpful discussions.

(13) Since our preparation was completed, this compound has been reported, m.p. 205–206°, $[\alpha]_D$ +28°, see ref 4c.

New Compounds

Some Substituted Pyrido(2,3)pyrazines

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Received September 30, 1963

Several pyrido(2,3)pyrazines have been prepared by condensing 2,3-diaminopyridine or 2,3,6-triaminopyridine with either an α-diketone or ethyl oxalate; one product, lead 6-aminopyrido-(2,3)-pyrazine-2,3-dicarboxylate, was obtained *via* permanganate oxidation of 6-amino-2,3-dimethylpyrido(2,3)pyrazine.

Experimental

All melting points are corrected. 2,3-Diaminopyridine, m.p. 112–113°, was prepared by Raney nickel reduction[2] of 2-amino-5-chloro-3-nitro-pyridine[3] and purified by sublimation at about 130° (17 mm.). The oxalate and hydrochloride salts of 2,3,6-triaminopyridine were prepared by reducing 2,6-diamino-3-nitrosopyridine with hydrogen sulfide. The nitroso compound was obtained by nitrosating 2,6-diaminopyridine dihydrochloride with sodium nitrite in aqueous acetic acid. The over-all procedure was a combination of the methods of Titov[4] and Engelman.[5] 6-Amino(2,3)-pyrazine, obtained in 44% yield from the reaction of diacetyl and 2,3,6-triaminopyridine, melted at 272–273° after recrystallization from water; a melting point above 250° has been reported by Korte.[6]

6-Acetamido-2,3-dimethylpyrido(2,3)pyrazine.—The amide was prepared by heating 6-amino-2,3-dimethylpyrido(2,3)-pyrazine[7] (m.p. 231–231.5°) with acetic anhydride and recrystallized twice from isopropyl alcohol. The white fluffy product collapsed at 179–180.5° to a yellow semisolid mass which turned orange-red at 187° and melted, forming a red liquid, at 200°. Further recrystallization did not change the melting characteristics.

Anal. Calcd. for $C_{11}H_{12}N_4O$: C, 61.04; H, 5.59; N, 25.91. Found: C, 61.01; H, 5.63; N, 25.80.

Lead 6-Amino(2,3)pyrazine-2,3-dicarboxylate.—A solution of 6.7 g. of potassium permanganate in 100 ml. of hot water was added dropwise, with stirring, over a period of about 150 min. to a refluxing solution of 1.74 g. (0.01 mole) of 6-amino-2,3-dimethylpyrido(2,3)pyrazine in 67 ml. of water containing 8 ml. of 10% aqueous sodium hydroxide. The precipitate of manganese dioxide was removed by filtration and the yellow filtrate neutralized with dilute nitric acid. An aqueous solution containing 12 g. of lead acetate trihydrate was added. After standing in an ice bath for several hr., the mixture was filtered and the yellow powder washed well with water. The salt weighed 4.80 g. (74%) and did not melt below 300°.

Anal. Calcd. for $C_9H_4N_4O_4Pb$: C, 16.72; H, 0.62; N, 8.67; Pb, 64.09. Found: C, 16.85; H, 0.68; N, 8.78; Pb, 64.04.

2,3-Dihydroxypyrido(2,3)pyrazine.—A mixture of 0.437 g. (0.004 mole) of 2,3-diaminopyridine and 5 ml. of ethyl oxalate was heated under reflux for 2 hr. Ether was added and the mixture filtered. The brown powder, after washing with ether, was dissolved in hot dilute aqueous sodium hydroxide and the solution was treated with decolorizing carbon. On neutralizing the filtrate with dilute hydrochloric acid to pH 7, its pink color

(1) The author wishes to thank the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service, for support of this project with a research grant (No. C-1642).

(2) J. B. Ziegler, *J. Am. Chem. Soc.*, **71**, 1891 (1949).

(3) J. R. Vaughan, Jr., J. Krapcho, and J. P. English, *ibid.*, **71**, 1885 (1949).

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(5) M. Engelman, U. S. Patent 2,136,044 (November 8, 1938); *Chem. Abstr.*, **33**, 1348 (1939).

(6) F. Korte, *Chem. Ber.*, **85**, 1012 (1952).

(7) J. Bernstein, B. Stearns, E. Shaw, and W. A. Lott, *J. Am. Chem. Soc.*, **69**, 1151 (1947).