Steroidal Dihydro-1,3-oxazines as Antitumor Agents

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Received July 24, 1961

Dihydro-*m*-oxazines were prepared by condensation of hexestrol, stilbestrol, equilenin and 17α-ethynylestradiol with formaldehyde and primary amines. Estrogenic activity was retained only by the products derived from 17α-ethynylestradiol, where some members also showed maximum inhibition of experimental adenocarcinoma EO771 in mice. Condensation of 3, 17, 20, 23 and 24-aminosteroids with formaldehyde and phenols gave dihydro-*m*-oxazines with steroidal *N*-substituents. Moderate inhibition of EO771 tumors was found in each class of steroids. The *N*-(3α,7α,12α-trihydroxycholan-24-yl)-dihydro-*m*-oxazines demonstrated bacteriostatic and antifungal activity *in vitro*.

In the preceding paper[2] it was demonstrated that dihydro-*m*-oxazines in general show a specific antitumor activity, unimpaired by the high toxicity usually found with carcinostatic agents. In an effort to enhance the specificity of this activity, the dihydro-*m*-oxazine moiety was attached to compounds with distinct biological functions. It was thought that such a linkage could provide a transport system which would direct the dihydro-*m*-oxazine function to selected target sites.

The importance of hormonal therapy in human malignancies such as prostatic and mammary tumors suggested the steroidal skeleton as a suitable component of the proposed biologically bifunctional molecules. When we found that the test system most responsive to dihydro-*m*-oxazines, adenocarcinoma EO771 in C57/Bl mice was moderately[3] inhibitied by some estrogenic compounds (hexestrol, 17α-ethynylestradiol and its methyl ether but not by stilbestrol, estradiol or estrone) and by the androgen 9α-fluoro-11β-hydroxy-17α-methyltestosterone, the rationale for a synthesis and biological testing of steroidal dihydro-*m*-oxazines was further strengthened.

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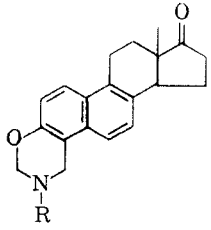
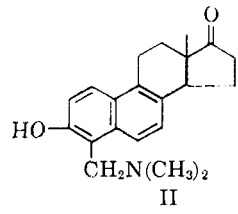
(2) M. E. Kuehne and E. A. Konopka, *J. Med. Pharm. Chem.*, **5**, 257 (1962).

(3) See reference 2 for biological test method and evaluation ratings.

Attachment of dihydro-*m*-oxazines to a steroidal carrier was accomplished by fusion of the two ring systems and by use of steroids as *N*-substituents on dihydro-*m*-oxazines. Thus estrogenic compounds were utilized to synthesize members of the first class and androstane, pregnane, cholane and norcholane residues for the *N*-substituted heterocycles.

Equilenin was condensed with formaldehyde and four primary amines to give the dihydro-*m*-oxazines Ia-d, in analogy with the products derived from β-naphthol.[2] Since it has been shown[2] that antitumor activity of dihydro-*m*-oxazines is often retained by the aminophenols obtained on hydrolytic cleavage of those heterocyclic compounds, the analogous Mannich condensation product II was also prepared from equilenin, with a large excess of formaldehyde and dimethylamine.





II

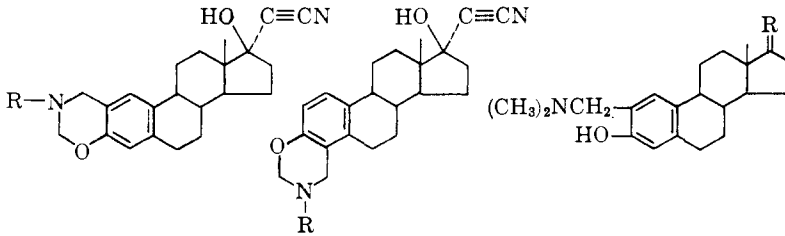
Ia, R = $CH_2C_6H_5$
b, R = C_6H_{11}
c, R = CH_3
d, R = $CH_2CH_2C_6H_5$

Condensation of the potent estrogen 17α-ethynylestradiol with formaldehyde and primary amines resulted in two series of isomeric dihydro-*m*-oxazines. Acid hydrolyses of the condensation products and colorimetric determination of formaldehyde support the expected dihydro-*m*-oxazine structures and rule out aminomethylene condensation products of the acetylenic group, which would still be compatible with the analytical data. Structural assignments to the chromatographically separated isomers were based on the expected preponderance of the 2,3-fused isomers IIIa-c over the 3,4-fused isomers IVa-c. In addition, it was found that each series showed a characteristic set of infrared absorption peaks and that those of the 2,3-fused isomers could be correlated with absorption bands of the Mannich condensation products Va,b of 17α-ethynylestradiol and estrone with formaldehyde and dimethylamine. The aminophenol Vb has

since been prepared by Patton[4] and rigorous structural assignment was obtained by its conversion to the known 2-methylestrone.

In the equilenin series, no significant inhibition of the E0771 tumor (see Table I) was found with the heterocyclic products Ia-d nor with equilenin methyl ether.[5] Slight, variable inhibition[3] of the tumor during the treatment period was produced by the Mannich condensation products II. Of the dihydro-*m*-oxazines, only the *N*-phenethyl compound Id showed slight estrogenic activity,[6] and this was also found in the aminophenol II.

Among the products derived from 17α-ethynylestradiol, good[3] inhibition of the E0771 tumor was found with the *N*-cyclohexyl 3,4-fused dihydro-*m*-oxazine IVb and moderate[3] inhibition with the *N*-phenethyl 2,3-fused compound IIIc and the Mannich product Vb. All compounds of this class (III, IV, V) are estrogenic at high doses.[6]



IIIa, R = $CH_2C_6H_5$
b, R = C_6H_{11}
c, R = $CH_2CH_2C_6H_5$

IVa, R = $CH_2C_6H_5$
b, R = C_6H_{11}
c, R = $CH_2C_6H_5$

Va, R = β-OH, α-C≡CH
b, R = =O

The condensation products of stilbestrol and hexestrol with formaldehyde and primary amines could neither be crystallized nor be converted to crystalline derivatives. While dihydro-*m*-oxazines were not obtained as completely pure entities, purification through the hydrochlorides, chromatography and precipitation as amorphous powders yielded materials with analytical data in rough agreement with bisdihydro-*m*-oxazine structures VIa-f and VIIa-f. None of these materials showed estrogenic activity, and moderate inhibition[3] of the E0771 tumor was found only with the reaction product VIIa, obtained from the condensation of hexestrol with cyclohexylamine and formaldehyde.

(4) T. L. Patton, *Chem. & Ind.*, 923 (1959); *J. Org. Chem.*, **25**, 2148 (1960).
(5) G. Sandulesco, W. W. Tchung, and A. Girard, *Compt. rend.*, **196**, 137 (1933).
(6) Estrogenic activity was determined through uterotropic response at 1–5 mg. per rat, administered subcutaneously for 3 days in ethanol–propylene glycol. The minimum effect dose was not established with these compounds.

TABLE I

INHIBITION OF ADENOCARCINOMA E0771 IN MICE, DURING (+) AND AFTER (*) TREATMENT[a]

Compound	7 Day Tumor	1 Day Tumor	Compound	7 Day Tumor	1 Day Tumor
Ia	−/−	−/−	f		−/−
b	−/−	−/−	VIII	−/−	
c	−/−	−/−	IX	++/***	
d	−/−	−/−	Xa	−/*	
II	±/−		b	−/−	
IIIa	−/−		c	++/**	
b	−/*		XIa	++/−	
c	++/***		b	−/−	
IVa	+/**		c	++/**	
b	+++/***		XIIa	+/**	
c	++/**		b	−/−	
Va	−/−		c	+/*	
b	+/**		d	−/−	
VIa		−/−	e	±+/**	
b		−/−	f	+/**	
d		++/*	XIIIa	−/−	
e		−/−	b	−/−	
f		−/−	c	+/*	
VIIa		++/*	XIVa		+/*
b		−/−	b		−/−
c		−/−	c		+/−
d		+/−	d		−/−
e		−/−	e	±+/**	

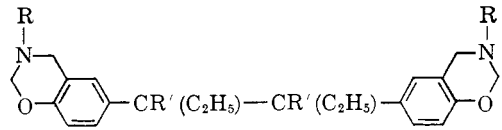
Compound	7 Day Tumor	1 Day Tumor
Estrone	−/−	
Estradiol	−/−	
17α-Ethynylestradiol	±/*	
17α-Ethynylestradiol-3-methyl ether	±/**	
Equilenin methyl ether	−/−	
Stilbestrol		−/−
Hexestrol	−/*	+/−
17β-Amino-5α-androstan-3β-ol	−/−	
9α-Fluoro-11β-hydroxy-17α-methyl-testosterone	+/*	
17β-Amino-5-androsten-3β-ol	+/*	
3β-Amino-5α-androstan-17β-ol	+/−	
20α-Amino-5α-pregnan-3β-ol	+/−	
20β-Amino-5α-pregnan-3β-ol	−/−	
20β-Amino-5-pregnen-3β-ol	−/−	
24-Amino-3α,7α,12α-trihydroxycholane	−/−	

TABLE II

ANTIMICROBIAL ACTIVITIES *in vitro*

Broth method: effective dilution, part per thousands, T.

	Compounds			
	XIVa	XIVc	XIVd	XIVe
Bacteria				
Staph. aureus	40 T	320 T	80 T	160 T
B. subtilis	80 T	640 T	320 T	320 T
E. Coli	5 T	10 T	5 T	5 T
Ps. aeruginosa	5 T	10 T	5 T	5 T
Fungi				
N. asteroides	>100 T	>100 T	>100 T	>100 T
Tr. mentagrophytes	>100 T	>100 T	1 T	>100 T
B. dermatitidis	>100 T	>100 T	50 T	50 T
H. capsulatum	>100 T	>100 T	50 T	>100 T
C. albicans	10 T	10 T	1 T	1 T
Cr. neoformans	10 T	1 T	1 T	1 T



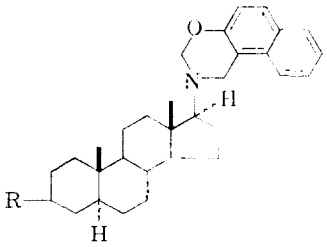
VI, R′ = —
VII, R′ = H

a, R = C_6H_{11}
b, R = $CH_2O_6H_5$
c, R = $CH_2CH_2C_6H_5$
d, R = 2-picolyl
e, R = 3-picolyl
f, R = CH_3

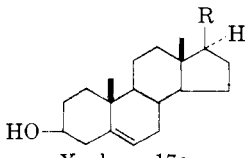
The steroidal dihydro-*m*-oxazines VIII, IX, Xa-c, XIa-c, XIIa-f, XIIIa-c, and XIVa-e were obtained by condensing β-naphthol, *p*-methoxyphenol, *p*-acetamidophenol, *p*-bromophenol and *p*-cresol with formaldehyde and the respective 3, 17, 20, 23 or 24-amino-steroids. Moderate and persistent growth inhibition[3] was produced by one derivative of each steroid class (IX, Xc, XIc, XIIe, XIVe).

The reported[7] interest in 24-amino-3α,7α,12α-trihydroxycholane and closely related compounds as antibacterial agents prompted a similar examination of our related dihydro-*m*-oxazines. The data in Table II indicate retention of this activity and antifungal activity in these new compounds. Antibacterial activity of the bromo-dihydro-*m*-oxazine XIVc exceeded that of 23-guanido-3α,7α,12α-

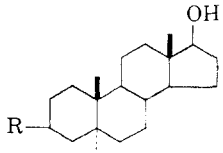
(7) S. P. James, F. Smith, M. Stacey, and M. Well, *J. Chem. Soc.*, 665 (1946).



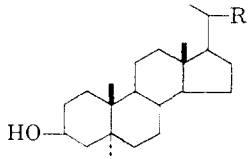
VIII, R = HO-
IX, R = HO...



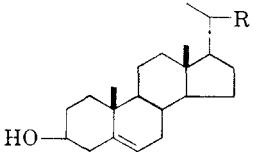
Xa, b, c = 17β



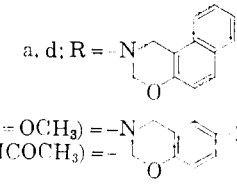
XIa, b, c = 3β



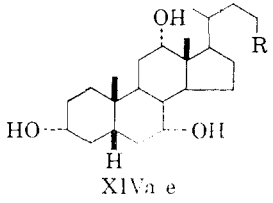
XIIa, b, c = 20β
d, e, f = 20α



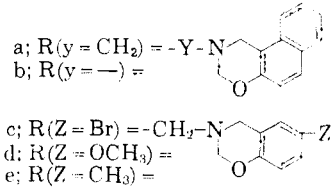
XIIIa, b, c = 20β



a, d; R = -N
b, e; R(Z = OCH_3) = -N
c, f; R(Z = $NHCOCH_3$) = -N



XIVa-e



a; R(y = CH_2) = -Y-N
b; R(y = —) =

c; R(Z = Br) = $-CH_2-N$
d; R(Z = OCH_3) =
e; R(Z = CH_3) =

trihydroxynorcholane, the best derivative previously reported.

Acknowledgment.—Spectral data and elemental analyses were obtained in our microanalytical laboratories. It is a pleasure to thank Mr. L. Dorfman for his help with analytical problems, Dr. C. R. Rehm for colorimetric formaldehyde determinations and Mr. B. Korzun and associates for paper chromatographic work. We thank Dr. J. Chart for determination of endocrine activities and Mr. J. Tanzola and Mr. T. Gilgunn for assistance with the antitumor,

bacteriostatic and antifungal screening. The studies were furthered by the continued interest and support of Dr. E. Schlittler.

Experimental[8]

17β-Amino-5α-androstan-3β-ol.—To a refluxing solution of 4.7 g. (15.4 mmoles) of 17-oximino-5α-androstan-3β-ol, [m.p. 184–186°; reported[9] 185–186°] in 130 ml. of propanol, 10.0 g. (0.43 mole) of sodium was added slowly, in portions. The cooled solution was poured into 3 l. of water, the amine collected by filtration and washed well with water. Several recrystallizations of the crude product[10] (4.0 g., m.p. 150–152°) from methanol and then from methylene chloride–hexane gave 2.8 g. (62% yield) m.p. 163–164° (reported,[11] 150° for the product of a Beckmann rearrangement of 20-oximino-5α-pregnan-3β-ol).

Anal. Calcd. for $C_{19}H_{33}NO$: C, 78.29; H, 11.41; N, 4.81. Found: C, 78.16; H, 11.44; N, 4.80.

2,3-Dihydro-2-(3β-hydroxy-5-androsten-17-yl and 3β-hydroxy-5α-androstan-17-yl and 3α-hydroxy-5α-androstan-17-yl)-1H-naph[1,2-e]-*m*-oxazine (Xa, VIII, IX).—To a cold solution of 0.38 g. (4.2 mmoles) of trioxymethylene and a trace of potassium hydroxide in 5 ml. of methanol was added 2.00 g. (6.2 mmoles) of (a) 17β-amino-5-androsten-3β-ol [m.p. 164–165°; reported[12] 160–162°] or (b) 17β-amino-5α-androstan-3β-ol [m.p. 163–164°] or (c) 17β-amino-5α-androstan 3α-ol [m.p. 184–186°; reported[12] 187–188°), respectively, dissolved in 40 ml. of methanol, followed by 9.10 g. (6.3 mmoles) of β-naphthol. The reaction mixtures were allowed to stand for 1 hr. at room temperature under an atmosphere of nitrogen and then refluxed for 90 min. With the 3 β-hydroxysteroids the product began to crystallize during the initial 10 min.; the 3α-hydroxy compound crystallized on chilling of the refluxed reaction mixture, giving (a) 2.40 g. (79% yield), m.p. 223–224°, on recrystallization from methylene chloride–ethanol.

Anal. Calcd. for $C_{31}H_{39}NO_2$: C, 81.36; H, 8.59; N, 3.06. Found: C, 81.05; H, 8.71; N, 3.22.

(b) 2.56 g. (84% yield), m.p. 227–228°, recrystallized from methylene chloride–ethanol.

Anal. Calcd. for $C_{31}H_{41}NO_2$: C, 81.00; H, 8.99; N, 3.05. Found: C, 80.96; H, 9.03; N, 3.02.

(c) 1.65 g. (54% yield), m.p. 175–176°, recrystallized from methylene chloride-methanol.

Anal. Calcd. for $C_{31}H_{41}NO_2$: C, 81.00; H, 8.99; N, 3.05. Found: C, 80.58; H, 9.24; N, 3.29.

Ultraviolet absorption of products (a), (b), (c): $\lambda_{max}^{methanol}$ 229 mμ (log ε 4.81

(8) Melting points are corrected.

(9) L. Ruzicka, M. W. Goldberg, J. Meyer, H. Brüngger, and J. Eichenberger, *Helv. Chim. Acta*, **17**, 1395 (1934).

(10) For stereochemical assignment of 17-aminosteroids see W. C. Shoppee and J. C. P. Sly, *J. Chem. Soc.*, 345 (1959).

(11) J. Schmidt-Thomé, *Chem. Ber.*, **88**, 895 (1955).

(12) L. Ruzicka and M. W. Goldberg, *Helv. Chim. Acta*, **19**, 107 (1936).

$_{287m\mu(\log}$ ϵ 3.61), 277 mμ (log ϵ 3.71), 289 mμ (log ϵ 3.62), 321 mμ (log ϵ 3.37), 333 mμ (log ϵ 3.35).

6-Acetamido- and 6-methoxy-2,4-dihydro-3-(3β-hydroxy-5-androsten-17-yl)-1,3-benzoxazine (Xc, Xb).—A trace of potassium hydroxide and 0.90 g. (10.0 mmole) of trioxymethylene were dissolved with warming in 75 ml. of anhydrous propanol, the solution cooled in ice and 4.80 g. (15.0 mmoles) of 17β-amino-5-androsten-3β-ol added, then (a) 1.86 g. (15.0 mmoles) of *p*-methoxyphenol or (b) 2.27 g. (15.0 mmoles) of *p*-acetamidophenol. The solutions were refluxed under an atmosphere of nitrogen for 15 hr. and then concentrated to dryness in vacuum. (a) The crude product was partially dissolved in 70 ml. of benzene and 1.6 g. of crystalline material, m.p. 210 or 230°, removed by filtration; this material showed no ultraviolet absorption, gave no melting point depression with that obtained when other phenols were used in the reaction, showed very poor behavior on recrystallization with persistent decrease in m.p. and is assumed to be the methylene diamine formed by condensation of one equivalent of formaldehyde and two equivalents of the steroidal amine. The benzene soluble portion was chromatographed on 60 g. of alumina (Woelm, basic, activity II). Eluting with benzene and crystallizing from ether, 2.2 g. of product, m.p. 137–143°, was obtained. After several recrystallizations from methylene chloride-heptane the material was rechromatographed on 50 g. of alumina (Woelm, basic, activity II) and eluted with 2:1 benzene:petroleum ether giving 2.0 g. (28% yield), m.p. 159–160°, after recrystallization from ether.

Anal. Calcd. for $C_{28}H_{39}NO_3$: C, 76.85; H, 8.98; N, 3.20. Found: C, 76.61; H, 9.10; N,3.25.

Ultraviolet absorption: $\lambda_{max.}^{methanol}$ 226 mμ (log ϵ 3.81), 293 mμ (log ϵ 3.58), $\lambda_{min.}^{methanol}$: 218 mμ (log ϵ 3.75), 253 mμ (log ϵ 2.45). (b) The reaction product was suspended in 50 ml. of benzene, the insoluble material filtered off and recrystallized from methanol, giving 1.7 g. of material, m.p. 237–243°. Digestion with 250 ml. of chloroform and recrystallization of the insoluble portion from ethanol gave 0.90 g. (12% yield) of product, m.p. 253–254°.

Anal. Calcd. for $C_{29}H_{40}N_2O_3$: C, 74.96; H, 8.68; N, 6.03. Found: C, 74.82; H, 8.79; N, 6.02.

Ultraviolet absorption: $\lambda_{max.}^{methanol}$ 250 mμ (log ϵ 4.12), 289 mμ shld. (log ϵ 3.45), $\lambda_{min.}^{methanol}$ 224 mμ(log ϵ 3.65). Alternative attempts at these reactions using methanol, dioxane or *tert*-butanol and other conditions gave poorer yields.

2,3-Dihydro-2-(17β-hydroxy-2-androstan-3β-yl)-1H-naphth[1,2-e]-*m*-oxazine (XIa).—To a solution of 0.41 g. (4.6 mmoles) of trioxymethylene and a trace of potassium hydroxide in 27 ml. of methanol was added 2.0 g. (6.9 mmoles) of 3β-amino-5α-androstan-17β-ol, m.p. 168–170° (reported[13] m.p. 170–171°) and 0.99 g. (6.9 mmoles) of β-napthol. After standing under nitrogen for 1 hr. at room temperature and refluxing an additional 30 min., the cooled reaction mixture was filtered, giving 2.4 g. of product, m.p. 208-209°; recrystallized from benzene, to 1.6 g., m.p. 209-210°; 50% yield.

Anal. Calcd. for $C_{31}H_{41}NO_2$: C, 81.00; H, 8.99; N, 3.05. Found: C, 80.81; H, 8.93; N, 3.04.

(13) J. Joska and F. Šorm, *Collection Czechoslov. Chem. Commun.*, **21**, 754 (1956).

6-Acetamido and 6-methoxy-2,4-dihydro-3-(17β-hydroxy-5α-androstan-3β-yl)-1,3-benzoxazine (XIc and XIb).—To a solution of 0.63 g. (7.0 mmoles) of trioxymethylene and a trace of potassium hydroxide in 25 ml. of dry propanol was added 3.00 g. (10.3 mmoles) of 3β-amino-5α-androstan-17β-ol, followed by (a) 1.56 g. (11.2 mmoles) of *p*-acetamidophenol or (b) 1.28 g. (10.3 mmoles) of *p*-methoxyphenol. After refluxing under nitrogen for 18 hr., the solvent was removed in vacuum. From (a) on crystallization with methanol–ether, 1.3 g. of dihydro-*m*-oxazine was obtained, m.p. 143–145°; yield 27%.

Anal. Calcd. for $C_{29}H_{42}N_2O_3$: C, 74.64; H, 9.07; N, 6.00. Found: C, 74.93; H, 9.06; N, 5.99.

A sample recrystallized from methylene chloride–benzene retained benzene, m.p. 208-209°.

Anal. Calcd. for $C_{35}H_{48}N_2O_3$: C, 77.19; H, 8.89; N, 5.14. Found: C, 76.51; H, 8.90; N, 4.97.

From (b) on trituration with cyclohexane 4.0 g. of amorphous solid was obtained and chromatographed on 30 g. of alumina (Woelm, neutral, activity II) in benzene. The initial eluate was crystallized from methylene chloride–heptane, giving 1.42 g., m.p. 133–134°; yield 32%.

Anal. Calcd. for $C_{28}H_{41}NO_3$: C, 76.49; H, 9.40. Found: C, 76.50; H, 9.52.

2,3-Dihydro-2-(3β-hydroxy-5α-pregnan-20α- and 20β-yl and 5-pregnen-20β-yl)-1H-naph[1,2-e]-*m*-oxazine (XIIa, XIId, XIIIa).—To a cold solution of 0.19 g. (2.1 mmoles) of trioxymethylene and a trace of potassium hydroxide in 5 ml. of methanol was added (a) 1.00 g. (3.15 mmoles) of 20α-amino-5α-pregnan-3β-ol [m.p. 171–173°; reported[14] 174–175°] in 25 ml. of methanol or (b) 1.00 g. (3.15 mmoles) of 20β-amino-5α-pregnan-3β-ol [m.p. 172–174°; reported[14] 174–176°) in 15 ml. of methanol or (c) 1.00 g. (3.15 mmoles) of 20β-amino-5-pregnen-3β-ol [m.p. 170–171°; reported[14] 170–172°] in 10 ml. of methanol. After the addition of 0.45 g. (3.1 mmoles) of β-naphthol, the reaction mixture was refluxed under nitrogen for 2 hr. giving partial crystallization or oiling out of products from the hot solution. After chilling in ice and filtration there was obtained from (a) on recrystallization from methylene chloride–methanol 0.97 g. of product, 63% yield, m.p. 197–199° dec.

Anal. Calcd. for $C_{33}H_{45}NO_2$: C, 81.27; H, 9.30; N, 2.87. Found: C, 80.89; H, 9.24; N, 2.90.

Paper chromatographic analysis: R_f. 65 using 2:1 xylene: butanone saturated with formamide as mobile phase on W1 paper treated with 1:1 formamide:methanol and 1% benzoic acid. Detection was by ultraviolet.

(b) Yield, 1.20 g., (78%) m.p. 122–133°, which on repeated recrystallization from methylene chloride–methanol showed fluctuating melting points around 138–140° dec.

Anal. Calcd. for $C_{33}H_{45}NO_2$: C, 81.27; H, 9.30; N, 2.87. Found: C, 81.05; H, 9.53; N, 2.91.

Paper chromatographic analysis: R_f. 85 using the same system as for 20α-compound above. Fractional crystallizations or chromatography on alumina,

(14) R. A. Lucas, D. F. Dickel, R. L. Dziemian, M. J. Ceglowski, B. L. Hensle, and H. B. MacPhillamy, *J. Am. Chem. Soc.*, **82**, 5688 (1960).

(Woelm, neutral, activity *II*) using 1:1 benzene:petroleum ether did not change the characteristics of the product.

(c) Crude material, 1.4 g., m.p. 119–126°, recrystallized repeatedly from benzene–petroleum ether and methylene chloride–methanol gave 0.28 g. (18% yield) m.p. 188–189°.

Anal. Calcd. for $C_{35}H_{43}NO_2$: C, 81.60; H, 8.92; N, 2.88. Found: C, 81.69; H, 9.03; N, 2.62.

The material could be chromatographed without losses on alumina (Woelm, basic, activity **II**) using 11:4 benezene:petroleum ether. Chromatography of the mother-liquor material gave only traces of additional product. Ultraviolet absorption of products from (a), (b), (c): $\lambda_{max}^{methanol}$ 229 mμ (log ε 4.79), 268 mμ (log ε 3.62), 278 mμ (log ε 3.69), 289 mμ (log ε 3.60), 321 mμ (log ε 3.34), 333 mμ (log ε 3.42).

2,4-Dihydro-6-methoxy-3-(3β-hydroxy-5α-pregnan-20α and 20β-yl and 3β-hydroxy-5-pregnen-20β-yl)-1,3-benzoxazine (XIIb, XIIe, XIIIb).—To a cold solution of 0.38 g. (4.2 mmoles) of trioxymethylene and a trace of potassium hydroxide in 25 ml. of anhydrous propanol was added (a) 2.00 g. (6.30 mmoles) of 20α-amino-5α-pregnan-3β-ol or (b) 2.00 g. (6.30 mmoles) of 20β-amino-5α-pregnan-3β-ol, or (c) 2.00 g. (6.30 mmoles) of 20β-amino-5-pregnen-3β-ol, then 0.78 g. (6.30 mmoles) of *p*-methyoxyphenol. The reaction mixtures were refluxed under nitrogen for 15 hr., concentrated in vacuum to dryness and triturated with methanol. From (a) 1.7 g., m.p. 108–120° dec., of material was obtained and recrystallized repeatedly from methylene chloride–methanol to give 1.1 g. (31% yield), m.p. 125–127°.

Anal. Calcd. for $C_{30}H_{45}NO_3 \cdot CH_3OH$: C, 74.51; H, 9.88; N, 2.80. Found (dried at 25°): C, 74.51; H, 9.75; N, 3.33.

Anal. Calcd. for $C_{30}H_{45}NO_3$: C, 77.04; H, 9.70. Found (dried at 110°): C, 76.85; H, 9.79.

From (b), 1.30 g. of product, m.p. 100–105° dec. recrystallized from methylene chloride–methanol to give 1.1 g. (31% yield), m.p. 110–115° dec. This material is a partial methanolate; the pure compound, m.p. 155–156°, can be obtained by repeated crystallization from methylene chloride–heptane but reverts back to the solvated form by addition of methanol.

Anal. Calcd. for $C_{30}H_{45}NO_3$: C, 77.04; H, 9.70; N, 3.00. Found: C, 76.92; H, 9.75; N, 3.27.

From (c), 0.75 g. (21% yield) of product was obtained, after recrystallization from methylene chloride–methanol, with melting points fluctuating between 110 and 150°. A sharply melting methanol free sample (by analysis) could not be obtained even after chromatography on alumina (Woelm, basic, activity II), eluting the product over ten identical fractions with 3:2 benzene: petroleum ether, or by crystallization from other solvents, but methanol was lost by drying at 110°.

Anal. Calcd. for $C_{30}H_{43}NO_3$: C, 77.38; H, 9.31; N, 3.01. Found: C, 76.97; H, 9.47; N, 2.88.

Ultraviolet absorption of products (a), (b), (c): $\lambda_{max.}^{methanol}$ 227 mμ (log ε 3.83), 295 mμ (log ε 3.57).

6-Acetamido-2,4-dihydro-3-(3β-hydroxy-5α-pregnan-20α and 20β-yl and 3β-

hydroxy-5-pregnen-20β-yl)-1,3-benzoxazine (XIIc, XIIf, XIIIc).—To a solution of 0.39 g. (4.3 mmoles) of trioxymethylene and a trace of potassium hydroxide in 30 ml. of anhydrous propanol, 2.10 g. (6.6 mmoles) of (a) 20α-amino-5α-pregnan-3β-ol, or (b) 20β-amino-5α-pregnan-3β-ol or (c) 20β-amino-5-pregnen-3β-ol were added, followed by 1.00 g. (0.66 mmole) of *p*-acetamidophenol. The reaction mixtures were refluxed for 15 hr. under nitrogen, the crude products filtered from the cooled solutions, mother-liquors concentrated in vacuum and the residues triturated with ethanol to give further small quantities of product. Recrystallization from ethanol gave (a) 0.95 g. (29% yield) of material, m.p. 247–248°.

Anal. Calcd. for $C_{31}H_{46}N_2O_3$: C, 75.26; H, 9.37; N, 5.66. Found: C, 74.83; H, 9.49; N, 5.83.

(b) furnished 0.80 g. (24% yield), m.p. 245–247°.

Anal. Calcd. for $C_{31}H_{46}N_2O_3$: C, 75.26; H, 9.37; N, 5.66. Found: C, 75.00; H, 9.47; N, 5.60.

(c) gave 0.68 g. (21% yield) m.p. 249–250°.

Anal. Calcd. for $C_{31}H_{44}N_2O_3$: C, 75.57; H, 9.00; N, 5.69. Found: C, 75.17; H, 9.26; N, 5.35.

Ultraviolet absorption of products, (a), (b), (c): $\lambda_{max.}^{methanol}$ 250 mμ (log ε 4.12), 289 mμ shld. (log ε 3.46).

23-Amino-3α,12α-dihydroxynorcholane.—After 3 hr. at room temperature, a solution of 18.0 g. of 3α,12α-diacetoxynorcholic acid, m.p. 205–206° (reported[15] 206–207°), from acetylation[16] of deoxynorcholic acid, in 20 ml. of thionyl chloride, was evaporated to dryness in vacuum, some dry benzene added and then removed in vacuum. After trituration with petroleum ether the acid chloride was filtered off and added directly, with rapid stirring, to 50 ml. of liquid ammonia. After evaporation of the ammonia, the residue was washed well with water and recrystallized from ethyl acetate, giving 10.0 g. (56% yield), m.p. 201–202°.

Anal. Calcd. for $C_{27}H_{43}NO_5$: C, 70.25; H, 9.39; N, 3.03. Found: C, 70.49; H, 9.51; N, 2.92.

A solution of 9.0 g. (19.5 mmoles) of the amide diacetate in 90 ml. of dry tetrahydrofuran was added to 5.4 g. (0.15 mole) of lithium aluminum hydride in 900 ml. of dry tetrahydrofuran over 1 hr. After refluxing for 3 hr., 30 ml. of water was added cautiously, then some Filter-Cel, the suspension filtered and the solid washed well with ether. After concentrating *in vacuo*, the crude amine was dissolved in chloroform, an excess of dry hydrogen chloride added, the chloroform decanted and the residual amine hydrochloride recrystallized from ethanol–ether to give 7.2 g. (92% yield) of salt, m.p. 300–302° (reported[7,17] 306° or 283° for the product obtained by rearrangement of 7-deoxycholic acid hydrazide).

Anal. Calcd. for $C_{23}H_{41}NO_2 \cdot HCl$: C, 68.98; H, 10.58; N, 3.49. Found: C, 68.56; H, 10.29; N, 3.64.

2,3-Dihydro-2-(3α,7α,12α-trihydroxycholan-24-yl and 3α,12α-dihydroxynorcholan-23-yl)-1H-naph[1,2-e]-*m*-oxazine (XIVa, XIVb).—To a solution of (a) 1.00 g. (2.3 mmoles) of 24-amino-3α,7α,12α-trihydroxycholane hydrochloride

(15) T. Kazuno and T. Simizu, *J. Biochem. (Japan)*, **29**, 421 (1939).
(16) B. Whitman and E. Schwenk, *J. Am. Chem. Soc.*, **68**, 1865 (1946).
(17) W. T. Caldwell, *ibid.*, **61**, 3584 (1939).

[m.p. 284°; reported[18,19] 271°] or (b) 1.00 g. (2.5 mmoles) of 23-amino-3α,12α-dihydroxynorcholane hydrochloride (m.p. 300–302°) in 3 ml. of methanol, was added (a) 3.4 ml. (2.3 mmoles) or (b) 3.7 ml. (2.5 mmole) of 3.8% methanolic potassium hydroxide solution. Potassium chloride was filtered off and (a) 0.14 g. (1.6 mmoles) or (b) 0.15 g. (1.7 mmoles) of trioxymethylene, dissolved in 1 ml. of methanol with a trace of potassium hydroxide and (a) 0.34 g. (2.4 mmoles) or (b) 0.36 g. (2.5 mmoles) of β-naphthol was added. The solutions were refluxed under nitrogen for 1.5 hr., concentrated to dryness in vacuum, the residues dissolved in ethyl acetate, a little ether was added, and the precipitate was filtered. The clear solutions were concentrated to dryness in vacuum, anhydrous benzene was added and the oxazines were precipitated as hydrochlorides by dropwise addition of dry hydrogen chloride in benzene solution. The hydrochlorides were recrystallized by dropwise addition of a large amount of ether to a solution in a few ml. of methanol, (a) m.p. 171–174°.

Anal. Calcd. for $C_{36}H_{53}NO_4 \cdot HCl$: C, 72.08; H, 8.89; N, 2.33. Found: C, 71.08; H, 8.83; N, 2.26.

(b) m.p. 155–156°.

Anal. Calcd. for $C_{35}H_{51}NO_3 \cdot HCl$: C, 73.72; H, 9.20; N, 2.46. Found: C, 73.96; H, 8.98; N, 2.61.

For biological testing the hydrochlorides were converted back to the free bases by extraction with methylene chloride from a suspension in iced, dilute, aqueous sodium hydroxide, giving (a) 0.73 g. (56% yield) and (b) 0.70 g. (53% yield) of amorphous powders.

Ultraviolet absorption of products (a) and (b) as free bases: $\lambda_{max.}^{methanol}$ 229 mμ (log ε 4.77), 268 mμ (log ε 3.60), 278 mμ (log ε 3.68), 389 mμ (3.59), 320 mμ (log ε 3.33), 333 mμ (log ε 3.42).

6-Bromo, 6-methoxy and 6-methyl-2,4-dihydro-3-(3α,7α,12α-trihydroxycholan-24-yl)-1,3-benzoxazine (XIVc, XIVd, XIVe).—With stirring, 10.0 g. (23.0 mmoles) of 24-amino-3α,7α,12α-trihydroxycholan hydrochloride was combined with 13.0 ml. (23.2 mmoles) of methanolic potassium hydroxide, the precipitated potassium chloride filtered off and with cooling in ice, a solution of 1.40 g. (15.5 mmoles) of trioxymethylene and a trace of potassium hydroxide in 10 ml. of methanol were added, followed by (a) 4.00 g. (23.1 mmoles) of *p*-bromophenol, or (b) 2.88 g. (23.1 mmoles) of *p*-methoxyphenol, or (c) 2.50 g. (23.1 mmoles) of *p*-cresol. The solutions were refluxed under nitrogen for 15 hr., cooled, concentrated to dryness in vacuum and the residues chromatographed on 200 g. of alumina (Woelm, neutral, activity II) using as initial solvents for (a) 30 ml. of 1:1 methylene chloride:benzene; for (b) 30 ml. of benzene to which 5 ml. of petroleum ether was added after solution of the reaction residue; for (c) 200 ml. of benzene. After washing with 500 ml. of benzene and 250 ml. of 1:1 benzene: methylene chloride, the products began to elute with methylene chloride and were removed largely by 1% methanol in methylene chloride. Higher concentration of methanol later eluted more polar, phenolic material. (Note: 24-amino-3α,7α,12α-trihydroxycholane is not eluted from a similar column with 1% but readily with 10% meth-

(18) F. Wessely and W. Swoboda, *Monatsh. Chem.*, **82**, 437 (1951).

(19) H. Lettré and H. Ballweg, *Chem. Ber.*, **91**, 345 (1958).

anol in methylene chloride.) The three products could not be crystallized, but in each case all chromatogram fractions were found to have equivalent ultraviolet maxima, comparable in intensity to model compounds. Precipitation by addition of benzene solutions to cold petroleum ether gave: (a) 3.2 g. (24% yield).

Anal. Calcd. for $C_{32}H_{48}BrNO_4$: C, 65.07; H, 8.19; N, 2.36. Found: C, 65.35; H, 8.30; N, 2.32.

Ultraviolet absorption: $\lambda_{max.}^{methanol}$ 227 mμ (log ε 3.92), 285 mμ (log ε 3.29), 291 mμ shld. (log ε 3.27).

(b) 4.3 g. (34% yield).

Anal. Calcd. for $C_{33}H_{51}NO_5$: C, 73.16; H, 9.49; N, 2.59. Found: C, 73.25; H, 9.73; N, 2.65.

Ultraviolet absorption: $\lambda_{max.}^{methanol}$ 227 mμ (log ε 3.82), 293 mμ (log ε 3.59).

(c) 3.2 g. (26% yield).

Anal. Calcd. for $C_{33}H_{51}NO_4$: C, 75.38; H, 9.78; N, 2.66. Found: C, 75.43; H, 10.04; N, 2.66.

Ultraviolet absorption: $\lambda_{max.}^{methanol}$ 225 mμ shld. (log ε 3.77), 282 mμ (log ε 3.38), 287 mμ shld. (log ε 3.36).

3′-(Benzyl, cyclohexyl, methyl and phenethyl)-2′,4′-dihydro-17-keto-1,3,5(10),6,8-estrapentaeno[3,4-e]-1′,3′-oxazine (Ia, Ib, Ic, Id).—A trace of potassium hydroxide and 0.30 g. (3.3 mmoles) of trioxymethylene were dissolved in 2 ml. of methanol, the solution was cooled in ice and 5.0 mmoles of the respective amine (0.50 g. benzylamine, 0.49 g. cyclohexylamine, 0.15 g. methylamine in 10 ml. of methanol, 0.60 g. phenethylamine) added, then 1.30 g. (4.9 mmoles of equilenin and 75 ml. of methanol. The solutions were left under nitrogen for 1 hr. at 20°, then refluxed for 2 hr., cooled and the crystalline products filtered off. Concentration of the mother liquors yielded further small quantities of the oxazines. Recrystallization from methylene chloride–ethanol gave: (a) 1.50 g. (77% yield) of *N*-benzyl oxazine, m.p. 188–189°.

Anal. Calcd. for $C_{27}H_{27}NO_2$: C, 81.58; H, 6.85; N, 3.52. Found: C, 81.55; H, 6.99; N, 3.59.

(b) 1.60 g. (84% yield) of *N*-cyclohexyl oxazine, m.p. 192–193°.

Anal. Calcd. for $C_{26}H_{31}NO_2$: C, 80.17; H, 8.02; N, 3.60. Found: C, 79.94; H, 8.06; N, 3.60.

(c) 1.20 g. (76% yield) of *N*-methyl oxazine, m.p. 190-192°.

Anal. Calcd. for $C_{21}H_{23}NO_2$: C, 78.47; H, 7.21; N, 4.36. Found: C, 78.92; H, 7.32; N, 4.23.

(d) 1.70 g. (84% yield) of *N*-phenethyl oxazine, m.p. 148–149°.

Anal. Calcd. for $C_{28}H_{29}NO_2$: C, 81.72; H, 7.10; N, 3.40. Found: C, 81.83; H, 7.24; N, 3.64.

In each case the product could be chromatographed in benzene on 50 g. of alumina (Woelm, activity III, basic) without loss of material or change in melting points.

3′-Benzyl, cyclohexyl and phenethyl-2′,4′-dihydro-17α-ethynyl-17β-hydroxy-1,3,5(10)-estratrieno[2,3-e]- and [3,4-e]-1′,3′-oxazine (IIIa, b, c and IVa, b, c).—Addition of (a) 1.10 g. (10.1 mmoles) of benzylamine, or (b) 1.00 g. (10.1 mmoles) of cyclohexylamine or (c) 1.22 g. (10.1 mmoles) of phenethylamine to a cold solution of 0.65 g. (7.2 mmoles) of trioxymethylene and a trace of potassium hydroxide

in 35 ml. of propanol was followed by 3.00 g. (10.1 mmoles) of 17α-ethynylestradiol. After refluxing for 15 hr. under nitrogen, the reaction mixtures were concentrated to dryness in vacuum, the residues dissolved in 2:1 benzene: petroleum ether and chromatographed on alumina (Woelm, basic, activity II). (a) The bulk of the material (2.8 g.) which was contained in the initial fractions was dissolved in methylene chloride–heptane and crystallized on gradual evaporation of the solvent giving 1.4 g. of the 2,3-isomer, recrystallized to 1.1 g. (21% yield), m.p. 94–96°. A sample recrystallized repeatedly for analysis had m.p. 100–101°; R_f 25 in system A (below) and 50 in system B (below).

Anal. Calcd. for $C_{29}H_{33}NO_2 \cdot CH_2Cl_2$: C, 70.29; H, 6.89; N, 2.74; Cl, 13.83. Found: C, 70.31; H, 6.89; N, 2.74; Cl, 13.80.

Alternatively, the compound could be purified by precipitation from ether-heptane.

Anal. Calcd. for $C_{29}H_{33}NO_2$: C, 81.46; H, 7.78. Found: C, 80.77; H, 7.93.

Fractional precipitation from ether-heptane of the mother liquor material gave 0.4 g. (9% yield) of the 3,4-isomer. R_f 90 in system A, 85 in system B.

Anal. Calcd. for $C_{29}H_{35}NO_2$: C, 81.46; H, 7.78. Found: C, 81.38; H, 7.88.

(b) The initial eluate of 3.3 g. was dissolved in cyclohexane and chromatographed on 50 g. of Florisil. The first fractions were crystallized from heptane, giving 0.59 g. of material, m.p. 108–113°, plus 0.33 g. of gummy crystals and 0.74 g. of amorphous material. Further 0.51 g. of amorphous material was obtained by eluting with 1:1 benzene:cyclohexane. Recrystallization of the initial material gave 0.20 g. (4.7% yield) of 3,4-isomer; purest sample, m.p. 141–143°; R_f 35 in system A (below).

Anal. Calcd. for $C_{28}H_{37}NO_2$: C, 80.15; H, 8.89, N, 3.34. Found:[20] C, 79.83; H, 9.19; N, 3.24.

Amorphous material (2,3 isomer): 1.2 g. (28% yield); R_f 35 in system A (below) or 30 in system B (below).

Anal. Calcd. for $C_{28}H_{37}NO_2$: C, 80.15; H, 8.89; N, 3.34. Found:[20] C, 79.20; H, 9.03; N, 3.15.

(c) The first eluate fractions contained 2.2 g. of material which on solution in methylene chloride–heptane and slow evaporation gave 0.38 g. (8.6% yield) of 3,4-isomer, m.p. 141–142° on recrystallization; R_f 40 in system B (below).

Anal. Calcd. for $C_{30}H_{34}NO_2$: C, 81.59; H, 7.99; N, 3.18. Found:[20] C, 80.97; H, 8.19; N, 3.49.

Amorphous material (2,3 isomer), 1.8 g. (41% yield); R_f 40 in system B (below).

Anal. Calcd. for $C_{30}H_{34}NO_2$: C, 81.59; H, 7.99; N, 3.18. Found: C, 81.44; H, 8.12; N, 3.11.

Paper Chromatography.—System A uses as mobile phase $CHCl_3$ saturated with formamide, and system B 1:1 benzene: $CHCl_3$ and 2% pyridine, both on W1 paper

(20) The dihydro-*m*-oxazines derived from ethynylestradiol generally gave, on repeated analyses, values for C 0.5–1% lower than calculated as a result of tenacious binding of methylene chloride (see above isolation and analyses of *N*-benzyl compound as 1:1 complex with methylene chloride and without methylene chloride). Formaldehyde determinations and infrared absorption (lack of N-H or bonded phenolic O-H) and absence of change on refluxing with tenfold excess of formaldehyde support the dihydro-*m*-oxazine structures.

treated with 1:1 formamide:methanol and 1% benzoic acid. Detection was with $SbCl_3$ and $PtCl_4$. Ethynylestradiol had R_f 55 in system A and 60 in system B; detection with $SbCl_3$ only.

Infrared Absorption in Chloroform.—All compounds displayed sharp absorption at 3570 cm.$^{-1}$ (O—H) and 3280 cm.$^{-1}$ (C≡C—H); no broad absorption 2300–2700 cm.$^{-1}$ (no hydrogen bonded OH). Prominent differences between the minor 3,4-isomer and the preponderant 2,3-isomer: 1610 cm.$^{-1}$ *vs.* 1630 and 1580 cm.$^{-1}$; 1490 cm.$^{-1}$ *vs.* 1510 cm.$^{-1}$; no absorption 1390–1450 cm.$^{-1}$ *vs.* 1430 cm.$^{-1}$.

2-Dimethylaminomethyl-17α-ethynylestradiol and 2-Dimethylaminomethylestrone (Va, b).—(a) A trace of potassium hydroxide and 2.00 g. (22.2 mmoles) of trioxymethylene were dissolved in 15 ml. of propanol, 2.00 g. (6.7 mmoles) of ethynylestradiol was added, the solution refluxed under nitrogen and 3.00 g. (66.7 mmoles) of dimethylamine in 30 ml. of propanol added dropwise. After refluxing an additional hr., the reaction mixture was cooled, concentrated to dryness in vacuum, the residue dissolved in 1:2 benzene: cyclohexane and chromatographed on 45 g. of Florisil. With this solvent 0.62 g. (25% yield) was obtained on recrystallization from methylene chloride-heptane, m.p. 162–163°.

Anal. Calcd. for $C_{22}H_{31}NO_2$: C, 78.15; H, 8.84; N, 3.96. Found: C, 78.18; H, 9.03; N, 3.88.

(b) The reaction was repeated with 0.77 g. (2.8 mmoles) of estrone, 1.00 g. (11.1 mmoles) of trioxymethylene in 20 ml. of 1:4 benzene:ethanol and 1.50 g. (33.3 mmoles) of dimethylamine in 20 ml. of the same solvent. On concentration and crystallization from methylene chloride–ethanol, 0.41 g. (44% yield) was obtained; m.p. 167–168° (reported[4] 166–168°). From equivalent amounts of amine and formaldehyde, estrone was recovered unchanged.

Infrared absorption in chloroform: Both products at 1590 and 1630 cm.$^{-1}$ and broad absorption at 2300–2700 cm.$^{-1}$ (bonded OH). In addition, (a) at 3570 cm.$^{-1}$ (O—H), 3280 cm.$^{-1}$ (C≡C—H) and (b) at 1730 cm.$^{-1}$ (17—C=O).

Chromotropic acid tests: the steroidal oxazine (12–15 mg.) was dissolved in 4 ml. of 3% sulfuric acid and formaldehyde distilled out with steam at 110–140°, collecting 95 ml. of distillate. A chromotropic acid test in 80% sulfuric acid, using an aliquot portion and colorimetric comparison with known standards, showed that 80–90% of the calculated formaldehyde could be trapped. 2-Dimethylaminomethyl-17α-ethynylestradiol gave the expected blank determination.

4-Dimethylaminomethylequilenin (II).—A solution of 1.50 g. (5.65 mmoles) of equilenin in 22 ml. of a 4:1 ethanol–benzene mixture was combined with a solution of 2.11 g. (23.7 mmoles) of trioxymethylene and a trace of potassium hydroxide in 8 ml. of the same solvent. To the refluxing solution, under nitrogen, 5.22 g. (105 mmoles) of dimethylamine in 54 ml. of this solvent mixture was added dropwise. After refluxing an additional hr., the reaction mixture was concentrated and 0.42 g. of crude product collected. Chromatography in benzene over 6 g. of Florisil and recrystallization from methylene chloride-ethanol gave 0.20 g. (11% yield), m.p. 184–186°.

Anal. Calcd. for $C_{21}H_{23}NO_2$: C, 77.98; H, 7.79; N, 4.33. Found: C, 77.68; H, 7.89; N, 4.35.

Reaction of Stilbestrol and Hexestrol with Formaldehyde and Amines (VIa–f, VIIa–f).—Stilbestrol (5.00 g., 18.7 mmoles), or hexestrol (5.04 g., 18.7 mmoles)

were added to cooled solutions of 2.24 g. (26.7 mmoles) of trioxymethylene and 0.02 g. of potassium hydroxide in 18 ml. of methanol, to which these amines had first been added: (a) 3.70 g. of cyclohexylamine, (b) 4.10 g. of benzylamine, (c) 4.52 g. of phenethylamine, (d) 4.03 g. of 2-aminomethylpyridine, (e) 4.03 g. of 3-aminomethylpyridine (each 37.4 mmoles), or to a mixture of (f) 4.5 g. (37.4 mmoles) of 25% aqueous methylamine, 6.08 g. (75.0 mmoles) of 38% aqueous formaldehyde and 40 ml. of methanol. The methylamine reactions were refluxed for 90 min. and all other reactions for 30 min. After cooling, the reaction mixtures were poured into water, extracted with methylene chloride, the extracts concentrated to dryness and the residues passed in benzene over 20 g. of alumina (Woelm basic, activity II). After concentrating, the eluted material was dissolved in ether and treated with a slight excess of dry hydrochloric acid in ether. The hydrochlorides were collected by filtration, washed well with ether and added to ice cold dilute aqueous sodium hydroxide. Rapid extraction with ether and concentration gave amorphous white powders which were used for biological testing.

Anal. Calcd. for stilbestrol and (a) $C_{34}H_{46}N_2O_2$: N, 5.44. Found: N, 5.22. (b) $C_{26}H_{28}N_2O_2$: N, 5.28. Found: N, 5.00. (c) (gummy material, discarded); (d) $C_{34}H_{36}N_4O_2$: N, 10.52. Found: N, 9.78. (e) $C_{34}H_{36}N_4O_2$: N, 10.52. Found: N, 8.61. (f) $C_{24}H_{36}N_2O_2$: N, 7.40. Found: N, 6.21.

Anal. Calcd. for hexestrol and (a) $C_{34}H_{48}N_2O_2$: N, 5.42. Found: N, 5.24. (b) $C_{26}H_{40}N_2O_2$: N, 5.26. Found: N, 5.33. (c) $C_{38}H_{44}N_2O_2$: N, 5.00. Found: N, 4.56. (d) $C_{34}H_{38}N_4O_2$: N, 10.48. Found: N, 10.07. (e) $C_{34}H_{38}N_4O_2$: N, 10.48. Found: N, 8.63. (f) $C_{24}H_{32}N_2O_2$: N, 7.36. Found: N, 5.44.

Antineoplastic Agents. IV. Mannich Base Nitrogen Mustards (Part A)[1,2,3]

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Received August 9, 1961

Among the various types of alkylating agents which have received clinical trial the nitrogen mustards appear to offer the most promise

(1) See, G. R. Pettit, M. V. Kalnins, T. L. Liu, E. G. Thomas, and K. Parent, *J. Org. Chem.*, **26**, 2563 (1961), for the preceding contribution.

(2) Abstracted in part from the Master of Science Thesis submitted by J. A. Settepani to the Graduate School, University of Maine, June, 1961.

(3) This investigation was aided by Grants No. T-79A and T-79B from the American Cancer Society.