3-Cyclic Ethylene Acetal of 17α-Hydroxy-3,11-dioxo-4-etiocholenohydroxamic Acid (IV).—A stock solution of hydroxylamine was prepared: to a solution of 13.90 g. of hydroxylamine hydrochloride in 140 ml. of methanol was added portionwise a solution of sodium methoxide prepared from 4.6 g. of sodium and 100 ml. of methanol. The resulting mixture was cooled briefly and filtered from the precipitated sodium chloride. The ketal I (29.0 g.) was dissolved in 450 ml. of hot ethanol and then cooled to room temperature. A solution of sodium methoxide (prepared from 3.45 g. of sodium in 75 ml. of methanol) was added, and then 200 ml. of hydroxylamine stock solution (prepared as described above). The reaction mixture was stirred at room temperature overnight and concentrated to dryness under reduced pressure. The residue was dissolved in 2100 ml. of water and acidified to pH 4.5 by the addition of about 1200 ml. of saturated dihydrogen sodium phosphate solution. The resulting colorless precipitate was filtered, washed with water and dried. Crystallization from ethanol gave 19.6 g. (67.5% yield) of pure IV, m.p. 222–223° dec., $[\alpha]^{25}D$ +21° (*c*, 1.06), λ_{max}^{KBr} 2.86, 3.0, 3.05, 5.88 and 6.01 μ. The compound gave a strong purple coloration with ferric chloride.

Anal. Calcd. for $C_{22}H_{31}NO_6$: C, 65.16; H, 7.17; N, 3.45. Found: C, 65.23; H, 7.82: N, 3.66.

17α-Hydroxy-3,11-dioxo-4-etiocholenohydroxamic Acid (V).—To a solution of 7.0 g. of IV in 900 ml. of methanol was added 200 ml. of 4.5 *N* hydrochloric acid and the resulting solution was allowed to stand overnight at room temperature under nitrogen. It was concentrated to about 400 ml. under reduced pressure and then diluted with 1 l. of water. The resulting precipitate was filtered, washed with water and dried. Crystallization from ethanol gave 4.49 g. (72%) of V, m.p. 223.5–225.5° dec., $[\alpha]D$ +196° (*c*, 0.216), $\lambda_{max}^{C_2H_5OH}$ 238 mμ (ε 16,040), λ_{max}^{KBr} 2.95, 3.0, 5.87, 5.99 and 6.08 μ. The compound gave a strong purple coloration with ferric chloride.

Anal. Calcd. for $C_{20}H_{27}NO_5$: C, 66.46; H, 7.53; N, 3.88. Found: C, 66.56: H, 7.76; N, 3.94.

3-Cyclic Ethylene Acetal of O-Methyl-17α-hydroxy-3,11-dioxo-4-etiocholenohydroxamic Acid (VI).—Compound IV (810 mg.) was dissolved with some difficulty in 75 ml. of hot ethanol, and the solution was then concentrated to about 25 ml. by distillation. The resulting solution, cooled to room temperature, was treated with a 2 ml. aliquot of sodium ethoxide solution (2.5 g. of sodium in 100 ml. of ethanol) and then 0.16 ml. of methyl iodide. The reaction mixture was allowed to stand overnight at room temperature and was concentrated to dryness under reduced pressure. The residue was dissolved in ether–methylene chloride (3:1), the solution washed with water, dried (Na_2SO_4) and evaporated to dryness. Crystallization of the residue from ethanol gave 428 mg. (51%) of VI, m.p. 226–227° dec. Further crystallization from ethanol gave an analytical sample, m.p. 230–231° dec., $[\alpha]^{25}D$ +2° (*c*, 0.352), λ_{max}^{KBr} 2.95 and 5.87 μ (broad). The compound gave no coloration on treatment with ferric chloride.

Anal. Calcd. for $C_{23}H_{33}NO_6$: C, 65.85; H, 7.93; N, 3.34. Found: C, 65.86; H, 8.14; N, 3.39.

O-Methyl-17α-hydroxy-3,11-dioxo-4-etiocholenohydroxamic Acid (VII). (a) By Hydrolysis of the Ketal VI.—To a solution of 400 mg. of the ketal VI in 52 ml. of methanol was added 11.5 ml. of 4.5 *N* hydrochloric acid and the resulting mixture was allowed to stand overnight at room temperature under nitrogen. It was then concentrated to about 10 ml. under reduced pressure and diluted with 75 ml. of water. The solution was saturated with ammonium chloride and extracted with ether–methylene chloride (3:1); the extracts were washed with brine, dried (Na_2SO_4) and evaporated to dryness. Crystallization of the residue from methanol gave 187 mg. (52%) of VII, m. p. 219.5–222°. Crystallization from methanol gave an analytical sample, m.p. 224–225.5° dec., $[\alpha]^{25}D$ +166° (*c*, 1.06), $\lambda_{max}^{C_2H_5OH}$ 238 mμ (ε 15,700), λ_{max}^{KBr} 2.84, 3.05, 5.86, 5.91 and 6.02 μ. The compound gave no coloration with ferric chloride.

Anal. Calcd. for $C_{21}H_{29}NO_5$: C, 67.18; H, 7.79; N, 3.73. Found: C, 67.22; H, 7.65; N, 3.84.

(b) By Alkylation of V.—To a suspension of 1.80 g. of V in 36 ml. of anhydrous ethanol was added a 3.60 ml. aliquot of sodium ethoxide solution (3.40 g. of sodium in 100 ml. of ethanol). To this cooled pale yellow solution was added 3.0 ml. of methyl iodide and the reaction mixture was allowed to stand overnight at room temperature under nitrogen. The solvent now was removed under reduced pressure, the residue dissolved in methylene chloride, the solution washed with water, dried (Na_2SO_4) and evaporated to dryness. The residue was crystallized from methanol to give 1.52 g. (81%) of VII, m.p. 224–226.5° dec. The melting point was undepressed upon admixture with the sample prepared by method (a).

Acknowledgment.—We are indebted to Dr. A. Steyermark and his staff for the micro analyses, and to Dr. A. Motchane, Mr. S. Traiman and Dr. V. Toome for the infrared and ultraviolet spectra.

Steroids and Related Products. XVIII.[1] The Synthesis of 11β,12α-Dibromoprogesterone[2]

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The recent finding that 9,11-dihalogenated derivatives of steroid hormones exhibit interesting biological activities[4–6] seemed to call for an investigation of the effect of dihalogenation in positions 11 and 12 on hormonal potency. We were attracted toward this problem not only because of our interest in the effects of halogenation on the biological activities of steroid hormones[7a–d] but also because our laboratory has developed an efficient method for the preparation of 11,12-unsaturated steroids[8,9a,b] which represent suitable starting materials for the synthesis of 11,12-dihalogenated derivatives.[10]

As a first representative of 11,12-dihalogenated steroid hormones of the progesterone-corticoid group, we synthesized 11,12-dibromoprogesterone (VII), using the readily available 11-pregnene-3,20-dione (I)[8,9b,11a–c] as starting material.

The unsaturated diketone I was transformed with ethylene glycol and selenium dioxide[12a,b] to the crystalline 3-monoethylenedioxy derivative II, previously obtained in this laboratory, as an intermediate, in the amorphous state.[9b] Bromination in chloroform at low temperature, with one molecular equivalent of bromine,

(1) Paper XVII of this series: Ch. R. Engel, W. W. Huculak, and S. Rakhit, *Can. J. Chem.*, **40**, 921 (1962).

(2) The results reported in this paper were presented before the 5th Annual Meeting of the Canadian Federation of Biological Societies, Quebec, June, 1962 [*cf. Proc. Can. Fed. Biol. Soc.*, **5**, 26 (1962)].

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(4) C. H. Robinson, L. E. Finckenor, E. P. Oliveto, and D. Gould, *J. Am. Chem. Soc.*, **81**, 2191 (1959).

(5) H. Reimann, E. P. Oliveto, R. Neri, M. Eisler, and P. L. Perlman, *J. Am. Chem. Soc.*, **82**, 2308 (1960).

(6) C. H. Robinson, L. E. Finckenor, R. Tiberi, M. Eisler, R. Neri, A. Watnick, P. L. Perlman, P. Holroyd, W. Charney, and E. P. Oliveto, *J. Am. Chem. Soc.*, **82**, 4611 (1960).

(7) Compare: (a) Ch. R. Engel and H. Jahnke, *Can. J. Biochem. Physiol.*, **35**, 1047 (1957); (b) Ch. R. Engel and R. Deghenghi, *Can. J. Chem.*, **38**, 452 (1960); (c) R. Deghenghi and Ch. R. Engel, *J. Am. Chem. Soc.*, **82**, 3201 (1960); (d) Ch. R. Engel, R.-M. Hoegerle, and R. Deghenghi, *Can. J. Chem.*, **38**, 1199 (1960).

(8) G. Just and Ch. R. Engel, *J. Org. Chem.*, **23**, 12 (1958).

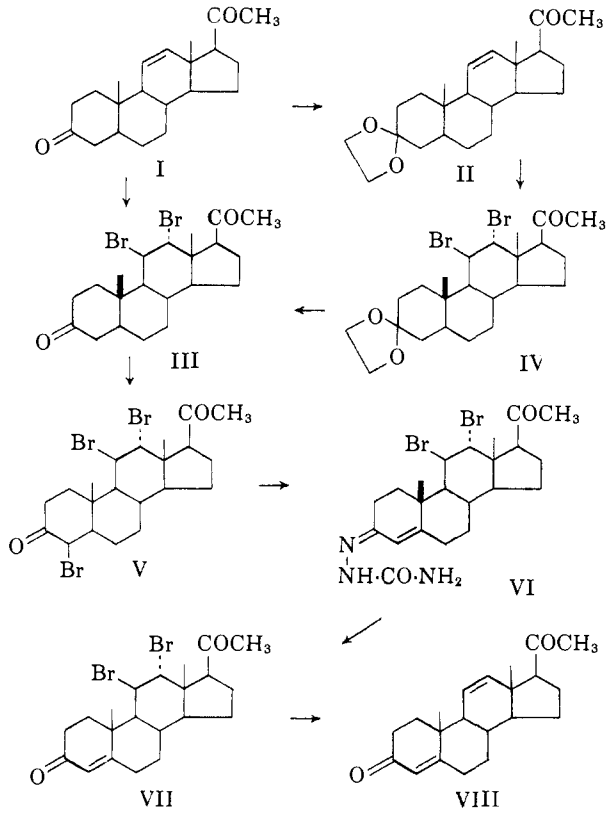
(9) Compare also (a) Ch. R. Engel, K. F. Jennings, and G. Just, *J. Am. Chem. Soc.*, **78**, 6153 (1956); (b) Ch. R. Engel and S. F. Papadopoulos, *J. Org. Chem.*, **26**, 2828 (1961).

(10) We sincerely thank Dr. D. Gould and his colleagues of the Schering Corporation, Bloomfield, N. J., for informing us, prior to publication, of the activities of their 9,11-dihalo steroids.

(11) (a) P. Hegner and T. Reichstein, *Helv. Chim. Acta*, **26**, 721 (1943); (b) J. von Euw and T. Reichstein, *ibid.*, **29**, 654 (1946); (c) A. Ruff and T. Reichstein, *ibid.*, **34**, 70 (1951).

(12) Compare (a) E. P. Oliveto, H. Q. Smith, C. Gerold, R. Rausser, and E. B. Hershberg, *J. Am. Chem. Soc.*, **78**, 1414 (1956); (b) E. P. Oliveto, C. Gerold, and E. B. Hershberg, *ibid.*, **76**, 6113 (1954).

gave the crystalline 11,12-dibromo ketal IV, which was converted without purification to the dibromo diketone III, by an exchange reaction with acetone in the presence of *p*-toluenesulfonic acid.[13,14] The same product was also obtained by direct bromination of the unsaturated diketone I, but in much lower yield.[15] Bromination of the dibromo diketone III, in acetic acid, gave the 4,11,12-tribromide V, which was selectively debrominated in positions 4/5, *via* the semicarbazone VI, by the method of McGuckin and Kendall,[16] thus affording the desired 11,12-dibromoprogesterone (VII).



The 11β,12α-configuration is assigned to the dibromides III, IV, VI, and VII, and to the tribromide V, in accordance with conformational rules[17] and in analogy with the findings of Turner; *et al.*[18], this assignment is confirmed by the facile debromination with sodium iodide in acetone, leading to the known 11-dehydroprogesterone (VIII).[8,11b,19,20] The 11α,12β-dibromide could not be isolated from the reaction products obtained by bromination of the unsaturated ketal II and of the unsaturated diketone I. Neither was it possible to isomerize 11β,12α-dibromoprogesterone (VII) by fusion, according to the procedure of Barton and King,[21] or by treatment with acid.

Drs. M. Eisler, S. Tolksdorf and P. L. Perlman of the Schering Corporation, Bloomfield, N. J., kindly tested 11,12-dibromoprogesterone (VII) intramuscularly in the Clauberg assay and found it to possess only one tenth the activity of progesterone. This low degree of progestational activity is not entirely surprising. Indeed, 11-non-oxygenated 12α-substituted progesterones have little or no luteoid activity. Thus, even in the presence of the activity enhancing 17α-methyl group, the introduction of 12α-hydroxy, 12α-acetoxy, and 12α-tosyloxy substituents results in inactivation of the parent compound.[22,23] On the other hand, the inactivity of 11,12-dibromoprogesterone is in marked contrast to the already mentioned progestational potency of 9,11-dihaloprogesterones[5] and to the activity of 12α-bromo-11β-hydroxy- and 12α-chloro-11β-hydroxyprogesterone.[24] The progestational inactivity of 11,12-dibromoprogesterone is also interesting from another point of view. Whereas the dibromide VII can be transformed readily, by chemical means, to the biologically active[20] 11-dehydroprogesterone (VIII), this transformation seems not to occur to an appreciable extent *in vivo* (nor is there any evidence for the reduction of the dibromide to progesterone). This parallels the already mentioned observation that 12α-tosyloxy-17α-methylprogesterone, which is readily converted by chemical means to the progestationally active 17α-methyl-11-dehydroprogesterone,[9a,22] exhibits no progestational activity. It also parallels the particularly striking finding by Julia, Plattner, and Heusser[25] that 5α,17β-dihydroxyandrostan-3-one, which is transformed chemically under exceedingly mild conditions to testosterone, exhibits only insignificant androgenic activity. It appears that steroids which undergo readily, by chemical means, a 1,2-elimination are, *in vivo*, generally not subject to the same transformation. Hence, the ready conversion of a steroid, by a chemical 1,2-elimination, to a hormonally active product, does in no way imply that it will be active and it seems obvious that a steroid should not be regarded or chosen as a potential "prohormone" (in the sense of a "provitamin"), merely because it can be converted by a chemical 1,2-elimination to a hormonally active derivative.

Dr. R. I. Dorfman, Worcester Foundation, Shrewsbury, Mass., kindly tested our 11,12-dibromoprogesterone for its anti-androgenic and anti-estrogenic activities. He found the product to possess only very low anti-androgenic activity in his chick-comb assay,[26] and to be devoid of anti-estrogenic activity in his mouse uterus assay.[27] Dr. Dorfman will publish the detailed results of these biological investigations separately.

(13) H. Schinz and G. Schäppi, *Helv. Chim. Acta*, **30**, 1483 (1947).

(14) The dibromo diketone III is very easily ketalized in position 3. Upon digestion of the product with methanol, at room temperature, a 3-monoketal is formed; this product is reconverted readily to the free diketone III.

(15) In this instance it was possible to isolate also an unsaturated tribromide, the constitution of which was not further investigated.

(16) W. F. McGuckin and E. C. Kendall, *J. Am. Chem. Soc.*, **74**, 5811 (1952).

(17) G. H. Alt and D. H. R. Barton, *J. Chem. Soc.*, 4284 (1954).

(18) R. B. Turner, V. R. Mattox, L. L. Engel, B. F. McKenzie, and E. C. Kendall, *J. Biol. Chem.*, **166**, 345 (1946).

(19) P. Hegner and T. Reichstein, *Helv. Chim. Acta*, **26**, 715 (1943).

(20) Ch. Meystre, E. Tschopp, and A. Wettstein, *ibid.*, **31**, 1463 (1948).

(21) D. H. R. Barton and J. F. King, *J. Chem. Soc.*, 4398 (1958).

(22) These biological results were obtained by Drs. M. Eisler, S. Tolksdorf, and P. L. Perlman with products synthesized in this laboratory [*cf.* footnote 9a and Ch. R. Engel, G. Just, and R. Buttery, *Can. J. Chem.*, **39**, 1805 (1961)].

(23) Recently, Just and Nagarajan [*Can. J. Chem.*, **39**, 548 (1961), and **40**, 377 (1962)] reported that 12-methyleneprogesterone, 12α-acetoxy-12β-methylprogesterone, and 12α-hydroxy-12β-methylprogesterone are progestationally inactive.

(24) J. Fried, W. B. Kessler, and A. Borman, *Ann. New York Acad. Sciences*, **71**, 494 (1958).

(25) S. Julia, Pl. A. Plattner, and H. Heusser, *Helv. Chim. Acta*, **35**, 665, 2080 (1952).

(26) "Methods in Hormone Research," R. I. Dorfman, Ed., Academic Press, New York, N. Y., 1962, Vol. II, pp. 315–323.

(27) *Ibid.*, Vol. II, pp. 113-126.

Experimental[28-30]

3-Ethylenedioxy-5β-pregn-11-en-20-one (II).—Following the procedure of Oliveto, *et al.*,[12a] 3 g. of 11-pregnene-3,20-dione (I) (m.p. 132–134°) was ketalized in 35 ml. of dichloromethane with 35 ml. of ethylene glycol and 3.5 g. of selenium dioxide, the reaction period being 36 hr. The crude reaction product (3.348 g.) was chromatographed on 52 g. of aluminum oxide (activity III). Petroleum ether–benzene (4:1) eluted 1.844 g. of ketal II, m.p. 102–104°, petroleum ether–benzene (1:1) 247 mg. of a mixture, and petroleum ether–benzene (1:4) 741 mg. of starting material I, m.p. 128–130°. Rechromatography of the intermediate fractions gave another 100 mg. of ketal II and 136 mg. of starting material I. Total yield of ketal II: 1.944 g. (62.2%); considering the recovery of starting material, the actual yield of the ketalization amounted to 90.3%. A sample of ketal II was recrystallized twice for analysis; colorless prisms, m.p. 103–104°, $[\alpha]^{23}D$ −28° (*c*, 1.000 in $CHCl_3$); ν_{max}^{KBr} 3005 $cm.^{-1}$ (C–H stretching, double bond), 1707 $cm.^{-1}$ (20-ketone); 1625 and 732 $cm.^{-1}$ (Δ^{11}-double bond), 1102 and 1080 $cm.^{-1}$ (3-ketal).

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 76.90; H, 9.53. Found: C, 76.73; H, 9.61.

11β,12α-Dibromo-3-ethylenedioxy-5β-pregnan-20-one (IV).—A solution of 1.181 g. of bromine in 14.48 ml. of absolute chloroform was added, with stirring, at −70°, to a solution of 2.64 g. of 3-ethylenedioxy-11-pregnen-20-one (II) (m.p. 99–100°) in 275 ml. of absolute chloroform. During the addition, which took 16 min., the temperature was maintained between −68 and −58°. The mixture was allowed to reach, within 22 min., −36°. The product was diluted with chloroform and the solution was washed with dilute, cold sodium thiosulfate solution and with water and was dried over sodium sulfate. Evaporation of the solvent *in vacuo* afforded 3.599 g. of crude dibromide IV, which crystallized upon moistening with ether–hexane; m.p. 112–116°; dec. p. 134°; positive halogen test; ν_{max}^{KBr} 1706 $cm.^{-1}$ (20-ketone), 1098 $cm.^{-1}$ (3-ketal). The product was converted, without further purification, to the dibromo diketone III (see below).

11β,12α-Dibromo-5β-pregnane-3,20-dione (III). (a) From the Dibromo Keto Ketal IV.—Deketalization of 3.59 g. of crude bromo keto ketal IV (see above) with 275 ml. of acetone and 275 mg. of *p*-toluenesulfonic acid, at room temperature in a carbon dioxide atmosphere,[18,31] gave 3.181 g. of crystalline material, m.p. 159–165° dec., $[\alpha]^{23}D$ +63°. Fractional crystallization from ether–hexane afforded 2.13 g. of dibromide III, m.p. 164–166° dec., $[\alpha]^{23}D$ +68–73°. The combined mother liquors (850 mg.) were again subjected to the deketalization reaction. The resulting 687 mg. of crystals, m.p. 154-163° dec., $[\alpha]^{22}D$ +50°, were recrystallized from ether–hexane to give another 170 mg. of dibromide III, m.p. 159–164° dec., $[\alpha]^{22}D$ +72.2°. The mother liquors (402 mg.) were chromatographed on 40 g. of silica gel. Benzene–ethyl acetate (97:3) eluted 171 mg. of a crystalline product, which, after one recrystallization from ether–hexane, melted at 142–144° dec. Benzene–ethyl acetate (95:5) eluted 93 mg. of crystals which afforded upon one recrystallization from ether 74 mg. of 11-pregnene-3,20-dione (I), m.p. 126–128°, identified by a mixture melting point and comparison of the infrared spectra. The chromatogram fractions (171 mg.) which had yielded the product melting at 142–144° dec., together with the adjoining fractions (total, 183 mg.) were rechromatographed on 16 g. of silica gel. Benzene–ethyl acetate (97:3) eluted 43 mg. of a crystalline product, which melted after one recrystallization from ether–hexane at 140–143°. Two further recrystallizations raised the m.p. to 149–150°; $[\alpha]^{23}D$ +5.8° (*c*, 1.000 in $CHCl_3$). *Anal.* Found: Br, 38.58. Further elutions with benzene–ethyl acetate (97:3) gave 60 mg. of another crystalline product, m.p. 116–120° dec. One recrystallization raised the m.p. to 124–126°; $[\alpha]^{23}D$ +84°. *Anal.* Found: Br, 26.45. Still further elutions with benzene–ethyl acetate (97:3) gave 86 mg. of the unsaturated diketone I. Considering the recovery of 11-pregnene-3,20-dione (I), the total yield of bromide III, from ketal II, amounted to 71.6%. A fraction of the dibromide III was recrystallized twice, from ether, for analysis; small prisms, m.p. 164–166° dec., $[\alpha]^{23}D$ +78.5° (*c*, 1.000 in $CHCl_3$); ν_{max}^{KBr} 1712 $cm.^{-1}$ (3-ketone), 1700 $cm.^{-1}$ (20-ketone).

Anal. Calcd. for $C_{21}H_{30}Br_2O_2$: C, 53.18; H, 6.38; Br, 33.70. Found: C, 53.50; H, 6.30; Br, 33.65.

(b) From 5β-Pregn-11-ene-3,20-dione (I).—A quantity of 1 g. of 5β-pregn-11-ene-3,20-dione (I) was brominated as described under (a) for ketal II. Crystallization of the amorphous reaction product from ether, afforded 200 mg. of dibromide III, m.p. 160–162° dec. The mother liquors were absorbed on 100 g. of silica gel. Elutions with benzene–ethyl acetate (97:3) gave 303 mg. of a crystalline product, melting with decomposition between 159 and 165°. One recrystallization afforded 219 mg. of a product melting at 165–166° dec. and representing an unsaturated tribromide (*vide infra*). Further elutions with benzene–ethyl acetate (97:3) gave 221 mg. of crude dibromide III. Benzene–ethyl acetate (95:5) eluted 174 mg. of impure starting material which gave, upon recrystallization, 159 mg. of starting material I, m.p. 128–130°. A sample of the tribromide was recrystallized twice, from ether, for analysis; fine small needles, m.p. 165–166° dec.; ν_{max}^{KBr} 1712 $cm.^{-1}$ (carbonyl), 1638 $cm.^{-1}$ (broad band of low intensity, unsaturation), 1000 $cm.^{-1}$ (C–O stretching ?); positive tetranitromethane test.

Anal. Calcd. for $C_{21}H_{27}Br_3O_2$: C, 45.76; H, 4.94; Br, 43.50. Calcd. for $C_{21}H_{29}Br_3O_2$: C, 45.59; H, 5.28; Br, 43.33. Found: C, 45.70; H, 4.92; Br, 43.36. This tribromide was not further investigated.

Considering the recovery of starting material I, the yield of dibromide III amounted to 23.7%.

4β,11β,12α-Tribromo-5β-pregnane-3,20-dione (V).—To a solution (1.85 g.) of 11β,12α-dibromopregnane-3,20-dione (III) (m.p. 164–166° dec.) in 85 ml. of acetic acid, a few drops of a 15% hydrogen bromide solution in acetic acid was added and, subsequently, dropwise with stirring, at room temperature, within 5 min., 640 mg. of bromine in 8.9 ml. of acetic acid. During the end of the addition, a crystalline material precipitated; it was separated by filtration, washed to neutral and dried. Thus, 861 mg. of crude, crystalline tribromide V, m.p. 165–168° dec., was obtained. Precipitation of the filtrate with water gave a second crop (1.103 g.) of crude crystalline tribromide V, m.p. 160–161° dec. (total yield of crude crystalline product 91%). Recrystallization from dichloromethane gave 1.27 g. of crystals, m.p. 172–173° dec., and 208 mg. of tribromide melting between 165 and 167° dec. (yield of purified tribromide V, 68.5%). A sample was recrystallized once from dichloromethane for analysis; very fine, small needles, m.p. 172–173° dec., $[\alpha]^{23}D$ +81° (*c*, 1.000 in $CHCl_3$).

Anal. Calcd. for $C_{21}H_{29}Br_3O_2$: C, 45.59; H, 5.28; Br, 43.33. Found: C, 45.45; H, 5.52; Br, 43.43.

11β,12α-Dibromo-4-pregnene-3,20-dione (11β,12α-Dibromoprogesterone) (VII).—According to the method of McGuckin and Kendall[15] and following a recently described procedure,[32] 1.25 g. of tribromide V (m.p. 172–173° dec.) was transformed in 50 ml. of chloroform and 71 ml. of *t*-butyl alcohol with 355 mg. of semicarbazide base to 1.18 g. of crystalline **semicarbazone VI**, m.p. 305–307° dec., λ_{max}^{EtOH} 234 mμ (log ε 4.08) and 270 mμ (log ε 4.86); when heated to 130°, the product darkens but lightens again at 190°. This product was converted with 159 ml. of acetic acid, 20.6 ml. of water and 7.5 ml. of 1.6 *N* pyruvic acid, to 860 mg. (85.4%) of crude **11β,12α-dibromoprogesterone (VII)**, m.p. 109–112° dec. The material was absorbed on 90 g. of silica gel. Elutions with benzene–ethyl acetate (97:3) afforded 70 mg. of a crystalline product, m.p. 171–174°, which did not represent an 11,12-dibromide and which was not further investigated. Further elutions with benzene–ethyl acetate (97:3 and 95:5) gave 590 mg. (58.6%) of pure 11β,12α-dibromoprogesterone (VII), m.p. 128–133° dec. A sample was recrystallized twice, from ether, for analysis. Very fine, short needles, m.p. 134–135° dec., $[\alpha]^{23}D$ +147° (*c*, 1.000 in $CHCl_3$), λ_{max}^{EtOH} 238 mμ (log ε 4.1); ν_{max}^{KBr} 1697 $cm.^{-1}$ (20-ketone), 1668 and 1622 $cm.^{-1}$ (Δ^4-3-keto doublet).

Anal. Calcd. for $C_{21}H_{28}Br_2O_2$: C, 53.40; H, 5.98; Br, 33.84. Found: C, 53.61; H, 6.14; Br, 33.76.

In another run, 200 mg. of tribromide V, m.p. 171–173° dec., was transformed in a similar manner to 110 mg. (64.4%) of pure 11,12-dibromoprogesterone VII, m.p. 132–134° dec.

Debromination.—To a solution of 74 mg. of 11β,12α-dibromoprogesterone (VII) (m.p. 134–135° dec.) in 10 ml. of acetone,

(28) All melting points were taken in evacuated capillaries and the temperatures are corrected.

(29) "Non-alkaline" aluminum oxide Woelm and Davison's silica gel No. 923 were used for chromatography.

(30) We are indebted to A. Bernhardt, Mülheim (Ruhr), Germany, for the microanalyses.

(31) Ch. R. Engel, *Can. J. Chem.*, **35**, 131 (1957).

(32) Ch. R. Engel, *J. Am. Chem. Soc.*, **78**, 4727 (1956).

there was added 100 mg. of sodium iodide and the mixture was refluxed in a nitrogen atmosphere for 6 hr. The usual working up[7a] afforded 70 mg. of a brownish oil which crystallized from ether–hexane. The product was filtered over 10 g. of aluminum oxide (activity II–III) affording 42 mg. (86% yield) of 11-dehydroprogesterone (VIII),[8,11b,19,20] m.p. 164–168°, identified by a mixture melting point with an authentic sample and by the comparison of its infrared spectrum with that of authentic material.

Negative Attempts of Isomerization of 11β,12α-Dibromoprogesterone (VII). (a)—A quantity of 50 mg. of 11β,12α-dibromoprogesterone (VII) was subjected for 30 min. to fusion, according to the procedure of Barton and King.[21] Chromatography on silica gel of the resulting product afforded 18 mg. of a crystalline material, m.p. 98–103°; $[\alpha]^{23}D$ +98° (*c*, 1.000 in $CHCl_3$), containing only little bromine (4.8%); ν_{max}^{KBr} 3450 cm.$^{-1}$ (very broad, associated hydroxy band), 1719–1710 cm.$^{-1}$ (broad carbonyl absorption). The product was not further investigated.

(b)—A solution of 60 mg. of 11β,12α-dibromoprogesterone (VII) in 20 ml. of *o*-xylene was refluxed for 4 hr. with 0.1 ml. of concd. hydrochloric acid and 0.03 ml. of water. The usual working up yielded 62 mg. of crude starting material, m.p. 110–116°, which melted upon one recrystallization at 126–128° dec.; $[\alpha]^{23}D$ +138° (*c*, 1.000 in $CHCl_3$). Other attempts of isomerization met with no more success.

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Potential Carcinolytic Agents Related to Cyclophosphamide[1]

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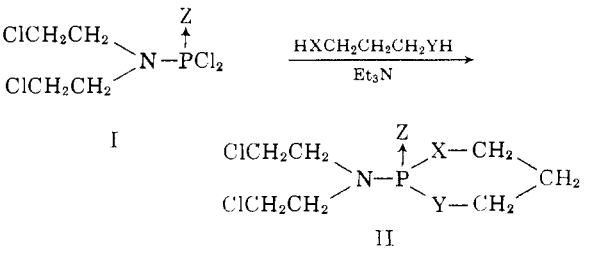
One of the early attempts at the development of enzyme-activated substrates for the much sought-after selectivity of drug action in cancer chemotherapy was the synthesis of N-phosphorylated nitrogen mustards.[3] These compounds, which are inactive precursors of cytotoxic mustards, could be activated by phosphamidases; and based on reports of abundance of these enzymes in some tumors it seemed possible for this "activation" to occur selectively in certain tumor cells. A number of phosphamide mustards in the original series[3] and in series synthesized subsequently[4,5] have shown remarkable selectivity of action in causing complete regression of many types of experimental tumors in animals with relatively little toxicity to the host.

In the case of cyclophosphamide IIa, the one compound tested clinically thus far, the results in humans are not nearly as dramatic as those in animals. Although this compound is not known to cause cures, it does produce beneficial effects in certain forms of human cancer and is one of the more effective drugs now in clinical use.

The mechanism of action of cyclophosphamide, which has been assumed to involve enzymatic "activation" by liberation of the bis(2-chloroethyl)amine (*nor*-nitrogen mustard) moiety, is still not clearly understood. In an attempt to gain insight into the nature of some structural features associated with the high activity of this compound and possibly to find related derivatives of even greater selectivity of action, we have synthesized a new series of derivatives II that are essentially isosteres of cyclophosphamide.

Compounds IIb and IIg were isolated as pure, crystalline products which were quite stable at low temperatures. In fact, the isosteres in which Z = O were all crystalline except IId which was prepared as a fairly pure but unstable oil. The isosteres in which Z = S were all oils; compound IIe was isolated in pure form, but was unstable even at low temperatures.



I

II

Ia, Z = O ((NSC-64119)[6]
Ib, Z = S (NSC-59505)

IIa, X, Z = O; Y = NH (NSC-26271)
IIb, X, Z = O; Y = S (NSC-65420)
IIe, Z = O; Y, Z = S (NSC-67105)
IId, X = NH; Y = S; Z = O (NSC-68118)
IIe, X = NH; Y, Z = S
IIf, X, Y = NH; Z = O
IIg, X, Y = S; Z = O (NSC-65422)
IIh, X, Y, Z = S

The remaining materials (IIe, IIf and IIh) were never obtained pure despite our intensive efforts. Attempts to purify the oily products by the usual techniques, including chromatography and molecular distillation at 10^{-6} mm. pressure, were all unsuccessful. Treatment with liberal amounts of activated carbon effected some improvement in purity as evidenced by analytical data.

After hydrolyzing cyclophosphamide with hydrochloric acid, one can account for about 85% of its *nor*-nitrogen mustard content by the γ-(4-nitrobenzyl)-pyridine (NBP) method.[7] However, when this method was used with the isosteres of cyclophosphamide and the intermediate phosphoramidic dichlorides (I) variable results (54% to 86% of *nor*-nitrogen mustard) were obtained depending on the nature of the isostere. Even when concentrated acid was used in an effort to inhibit destruction of the *nor*-nitrogen mustard during hydrolysis, the NBP method was not a useful analytical

(1) Cyclophosphamide: 2-[bis(2-chloroethyl)amino]-1,3,2-oxazaphosphorinane-2-oxide. This work was sponsored by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-4360.

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(3) O. M. Friedman and A. M. Seligman, *J. Am. Chem. Soc.*, **76**, 655, 658 (1954).

(4) O. M. Friedman, E. Boger, V. Grubbianskas and H. Sommer, *J. Med. Chem.*, **6**, 50 (1963).

(5) H. Arnold and F. Bourseaux, *Angew. Chem.*, **70**, 539 (1958); H. Arnold, F. Bourseaux and M. Brock, *Arzneimittel-Forsch.*, **11**, 143 (1961).

(6) The NSC accession numbers were assigned by the Cancer Chemotherapy National Service Center.

(7) O. M. Friedman and E. Boger, *Anal. Chem.*, **33**, 906 (1961).