Recrystallization from *n*-propyl alcohol did not change the decomposition range.

Anal. Calcd. for $C_{16}H_9NO_2$: C, 77.09; H, 4.44; N, 5.62. Found: C, 77.36; H, 4.51; N, 5.89.

2-Nitrosofluoranthen-3-ol.—Fluoranthen-3-ol[8] (2.0 g.) in absolute ethanol (50 ml.) at 0° was treated with concentrated hydrochloric acid (1 ml.) and *n*-butyl nitrite (0.5 g.) in ethanol (35 ml.). The mixture was stirred at 0° for 1 hr. and heated at 100° for 1.5 hr. The resulting brown precipitate after fractional crystallization from absolute ethanol and successive crystallizations from absolute ethanol and from benzene gave yellow-orange needles melting at 216° dec.; yield 0.135 g.

Anal. Calcd. for $C_{16}H_9NO_2$: C, 77.72; H, 3.67; N, 5.67. Found: C, 78.29; H, 3.83; N, 5.11.

2-Amino-1,2,3,10b-tetrahydrofluoranthen-3-one Hydrochloride (III).—2-Oximino-1,2,3,10b-tetrahydrofluoranthen-3-one (3.0 g.) was hydrogenated at 3.16 kg./cm.^{2} in absolute ethanol (100 ml.) containing 3 moles of HCl and 10% Pd-C (0.3 g.) for 1.5 hr. The resulting mixture was filtered and the amine hydrochloride was dissolved in hot water degassed previously with nitrogen. Cooling gave yellow crystals which after two further recrystallizations from hot water gave small white platelets melting at 151° dec.; yield 0.85 g.

Anal. Calcd. for $C_{16}H_{13}NO \cdot HCl$: C, 70.71; H, 5.19; N, 5.31. Found: C, 70.24; H, 5.31; N, 5.59.

Acknowledgment.—The authors wish to thank Dr. Richard McLean of Smith, Kline and French Laboratories for the pharmacological results.

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Pteridinosteroids. II. Synthesis of 17β-Acetoxy-5α-androstano[2,3-*g*]-2′,4′-diaminopteridine[1a,b]

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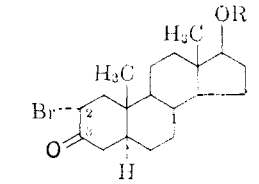
The synthesis and biological evaluation of pteridinosteroids, a new series of polycyclic compounds, was undertaken in our laboratories in the hope that such compounds may combine the antifolic activity of 2,4-diaminopteridines with lipid solubility and with the favorable cellular transport properties of steroid molecules. The first compound representing this series, a [4,3-*g*]-fused pteridinosteroid, was described recently and was shown to satisfy, qualitatively at least, the above expectations.[2]

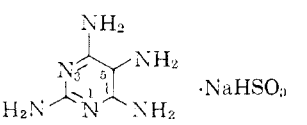
It was hoped that, if the fusion of the pteridine nucleus with respect to the A and B rings is linear, some of the hormonal activities of the steroid may be maintained, modified, or accentuated. (Examples for such effects have been seen in some other steroidally fused heterocycles.[3]) For this reason, the synthesis of a [2,3-*g*]-fused pteridinosteroid was undertaken.

The starting material for this synthesis, 17β-hydroxy-2α-bromo-5α-androstan-3-one (Ia), was prepared by direct bromination of 17β-hydroxy-5α-androstan-3-one (5α-dihydrotestosterone) in glacial acetic acid.[4] Infrared spectrum showed a shift of the carbonyl stretching band from 5.9 to 5.8 μ, indicating equatorial halogen (2α-Br).[5] This compound was acetylated and was carefully purified[6] in the form of the acetate (Ib).

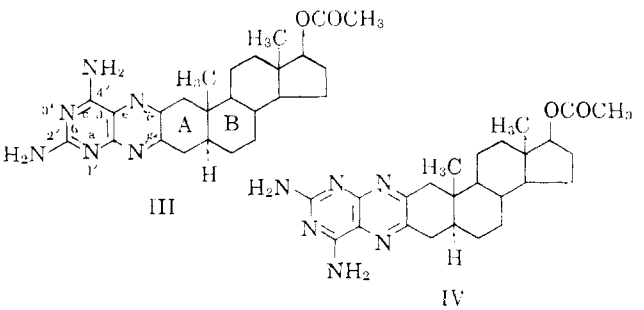
Condensation of Ib with 2,4,5,6-tetraaminopyrimidine bisulfite (II) to form a pteridine ring system, would be expected to require oxidative conditions, since the direct (anaerobic) condensation product would be a nonaromatic dihydropteridine. Therefore, the reaction was tried first in glacial acetic acid solution under a variety of oxidative conditions, including presence of iodine, passing air through the reaction mixture, etc. All of these methods caused the formation of dark-colored by-products which made the purification of the



Ia, R = H
Ib, R = $COCH_3$



II



III

IV

pteridinosteroid extremely difficult. Such by-products might arise from the oxidation and subsequent self-condensation of the tetraaminopyrimidine. This did not occur when the reaction was carried out with air oxidation in pyridine and in the presence of dicyclohexylcarbodiimide, but in this case the yield of the product was very low.

On the other hand, just as in the case of the classical folic acid synthesis,[7] condensation with concomitant oxidation to the pteridine occurred readily if the reactants were simply combined in an aqueous (alcoholic) solution and allowed to react at room temperature with stirring for an extended period of time. A series of experiments was carried out to establish the optimal pH; it was found that condensation at pH 7.5–8.0

(1) (a) This investigation was supported by PHS Research Grant No. CA-06695 and CA-03603, from the National Cancer Institute. (b) A preliminary report of this work was presented before the Division of Medicinal Chemistry, 145th National Meeting of the American Chemical Society, New York, N. Y., Sept., 1963, Abstracts, p. 28-O. (c) To whom inquiries should be directed.

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(6) The presence of trace amounts of impurities caused difficulties in the purification of the pteridinosteroid.

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TABLE I

RAT ANTIANDROGEN ASSAY

Compound	Daily dose,[a] γ	Levator ani, mg.	Seminal vesicle, mg.	Ventral prostate, mg.	% inhibition
Controls	...	8.8	1.8	9.0	
Testosterone	1 s.c.	9.2	3.2	14.5	
Testosterone	10 s.c.	11.3	7.0	24.3	
III	100 s.c.	10.8	2.8	9.5	
III	1[b] s.c.	9.8	2.5	8.3	
III + Testosterone	100 s.c. + 1 s.c.	10.0	3.5	14.5	
III + Testosterone	1[b] s.c. + 1 s.c.	10.5	3.3	13.7	
III	100 p.o.	9.8	2.5	9.3	
III	1[b] p.o.	8.3	2.0	7.5	
III + Testosterone	100 p.o. + 1 s.c.	7.5	2.0	10.0	82
III + Testosterone	1[b] p.o. + 1 s.c.	9.3	2.8	11.5	55

[a] S.c. = subcutaneous injection; p.o. = *per os*. [b] In mg.

gave the cleanest product. The condensation reaction was found to proceed for about 72 hr.; after this, no further increase of yield was observed.

Details of the purification procedure are given in the Experimental part. The analytically pure pteridinosteroid is a yellow crystalline solid which is soluble in alcohol and chloroform. It has the characteristic ultraviolet absorption spectrum of pteridines (two maxima, at 257 and 375 mμ) and optical activity due to the steroid portion of the molecule. Its infrared spectrum is almost identical with that of the [4,3]-fused pteridinosteroid[2] except for some minor differences in the fingerprint region.

Based on analogy with established reactions[7,8] the halogen is expected to react preferentially with the more basic 5-amino group of the pyrimidine, while the carbonyl group condenses with the imino-type 4- or 6-substituents (which are symmetrical); consequently, of the two possible position isomers, III and IV, the former represents the proposed structure of this compound.

In microbiological experiments using the *Lactobacillus leichmannii* system,[9] the new, linear polycyclic compound (III) appears to be more active as an antifolic agent than the [4,3-*g*]pteridinosteroid.[2] Half-maximal inhibition is obtained at 1-γ/ml. concentration, while approximately 5-γ/ml. concentration is required for the [4,3-*g*]pteridinosteroid.

Figure 1 shows the reversal of this inhibitory effect by folinic acid. The inhibition appears within this range to be of the competitive type with an inhibition index of approximately 3000.

In animal experiments, III showed no androgenic activity in the rat or chick, but it appears to be a somewhat more effective antiandrogen than the [4,3-*g*]-pteridinosteroid.[2] The results of the rat assay are shown in Table I. It is interesting to note that the compound is inactive on parenteral administration but shows substantial inhibition of testosterone if it is given orally. In the chick assay (Table II), the inhibition of testosterone stimulation of the chick comb averaged 50%.

(8) D. R. Seeger, D. B. Cosulich, J. M. Smith, Jr., and M. E. Hultquist, *J. Am. Chem. Soc.*, **71**, 1753 (1949).

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TABLE II

CHICK ANTIANDROGEN ASSAY

Compound	Daily dose,[a] γ	No. of chicks	Comb wt., mg.	% inhibition
Controls	...	15	18.4	
Testosterone	1	14	50.1	
Testosterone	10	14	81.7	
Testosterone enanthate	0.5[b]	15	74.5	
III	50	15	16.9	
III	500[c]	13	20.5	
III + Testosterone	50 + 1	15	33.3	53
III + Testosterone	500[c] + 1	14	38.0	38
III + Testosterone enanthate	500[c] + 0.5[b]	14	47.6	48

[a] Applied to comb locally. [b] Single intramuscular injection; in mg. [c] Suspension.

Due to insufficient material, antitumor testing of III was done only against Sarcoma 180 in mice, using intraperitoneal route of administration. The results were inconclusive. However, the above mentioned dependence of the antiandrogen activity of this compound on the route of administration indicates the need for additional testing in this tumor system.

Experimental[10]

17β-Hydroxy-2α-bromo-5α-androstan-3-one (Ia).—To a solution of 17β-hydroxy-5α-androstan-3-one (50 g.) in glacial acetic acid (200 ml.) was added rapidly a solution of bromine in glacial acetic acid (200 ml., 5.0% Br_2 in acetic acid v./v.). When the bromine color disappeared, the solution was diluted with water, and the precipitate was collected; recrystallization from acetone–petroleum ether, followed by aqueous methanol, gave Ia; m.p. 182–183° dec.; lit.[4] m.p. 180–181° dec.; yield, 42 g. (66%).

17β-Acetoxy-2α-bromo-5α-androstan-3-one (Ib).—One gram of Ia was dissolved in pyridine (2 ml.) and cooled in ice, while acetic anhydride (2 ml.) was added with stirring. After standing at room temperature for 6 hr., the solution was poured in a thin stream and under vigorous stirring into ice-cold water. The solids were filtered with suction and repeatedly washed with hot water, then dried in an oven at 110° for 4 hr. (900 mg.). It was dissolved in 5 ml. of benzene–petroleum ether (b.p. 30–60°) mixture (1:1) and chromatographed over a column of Woelm neutral

(10) Melting points were taken in a Kofler micro hot stage and are corrected. Microanalyses by Galbraith Laboratories, Inc., Knoxville, Tenn.

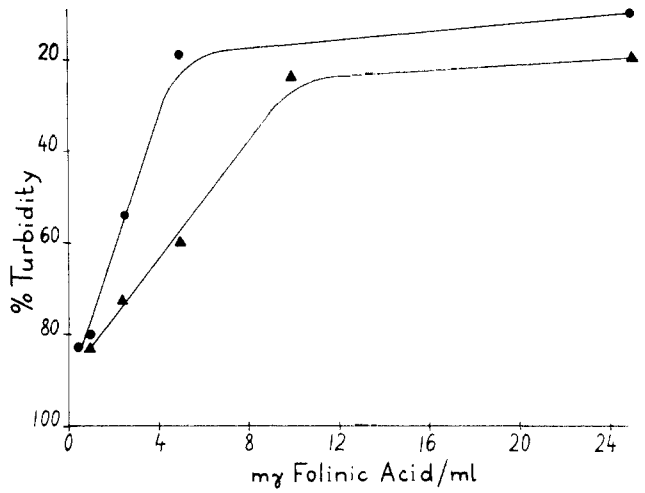

Fig. 1.—Reversal of inhibitory action of pteridinosteroid (III) by folinic acid on *L. leichmannii*: ●—●, III, 10 γ/ml.; ▲—▲, III, 20 γ/ml.

alumina (activity grade III, 15 g.). The product was eluted from the column with benzene (200 ml.) and crystallized as sharp needles from aqueous methanol, m.p. 176–178°; yield, 850 mg. (Ib). It gives one spot by thin layer chromatography (adsorbent, alumina; solvent, ether–alcohol, 9:1).

Anal. Calcd. for $C_{21}H_{31}BrO_3$: C, 61.31; H, 7.54; Br, 19.46. Found: C, 61.13; H, 7.50; Br, 19.30.

17β-Acetoxy-5α-androstano[2,3-*g*]-2′,4′-diaminopteridine (III).—A solution of 1 g. of Ib in methanol (150 ml., 80°, 65°) was kept well stirred while 2,3,4,6-tetraaminopyrimidine bisulfite[11] (II) (3 g.) was added in small portions during the course of 2 hr. The pH of this uniform suspension (6.3) was adjusted to 7.5–8.0 by dropwise addition of 0.5 *N* sodium hydroxide with vigorous stirring. A deep yellow solution resulted, and the reaction was allowed to proceed for 72 hr. at room temperature during which time an ultraviolet absorption peak appeared in the 360–370 mμ region and, at the end of this period, the ratio of absorbancies at 260 *vs.* 370 mμ reached a constant value between 3 and 5.

The solution was neutralized (pH 7) with glacial acetic acid and the dark red solids were filtered (to facilitate concentration of the solution). The filtrate was evaporated to dryness *in vacuo*; the residue was mixed with the dark solids that had been collected by filtration and with some Celite, then extracted in a Soxhlet apparatus with chloroform for 48 hr., the solvent being replaced freshly after every 12 hr. Thin layer chromatography of this extract on alumina with chloroform–ethanol (9:1) gave, on examination under ultraviolet light, an oblong fluorescent spot indicating that probably some deacetylation of the steroid had occurred during the condensation reaction. The combined chloroform extracts were evaporated to dryness and then dried further in a vacuum oven at 110° for 12 hr. (0.8 g.). The residue was dissolved in dry pyridine (10 ml.) and cooled in an ice bath while acetic anhydride (3 ml.) was added with vigorous stirring. The mixture was allowed to stand at room temperature for 6 hr. The solids were collected at the pump, dried (0.6 g.), and dissolved in a minimum volume of chloroform (15 ml.). Examination by thin layer chromatography, using alumina with chloroform–ethanol (9:1), showed only one well-defined spot. The chloroform solution of the pteridinosteroid was adsorbed over a column of Woelm basic alumina (activity grade III, 50 g.). Elution of the column with benzene (200 ml.) gave white solids (probably steroid). Elution with chloroform (500 ml.) gave fractions yielding a yellow residue which had an ultraviolet absorbancy ratio at 260/370 mμ of approximately 3.0. These fractions were combined and dissolved in chloroform (20 ml.) and addition of petroleum ether (b.p. 30–60°) (50 ml.) precipitated yellow solids, m.p. 250–300° dec. (200 mg.).

This was redissolved in benzene (20 ml.) and adsorbed over a column of neutral Woelm alumina (activity grade III, 25 g.). The chloroform–ethanol (9:1, 250 ml.) eluate fraction showed characteristic ultraviolet spectra of the pteridinosteroid. Crystallization from chloroform–petroleum ether (b.p. 30–60°) mixture (2:5 v./v.) gave yellow crystals, which did not melt below 250° (gradual decomposition) (170 mg.). The compound gave a positive Liebermann–Burchard reaction. Sodium fusion test for halogen and Zimmermann color reaction for 3-keto group were negative; $[\alpha]^{28}D$ +57° (*c* 1.0, $CHCl_3$); λ_{max}^{EtOH} 257 mμ (ε 25,600), 373 (9900). Infrared spectrum shows bands for NH_2, C=O, C=N, CH, CH_2, CH_3, and C—O—C. N.m.r. spectrum shows absorption for the angular methyl groups and for the acetyl group.

Anal. Calcd. for $C_{25}H_{34}N_6O_2$: C, 66.65; H, 7.60; N, 18.65. Found: C, 66.50; H, 7.54; N, 18.40.

Microbiological Assay.—The compound was dissolved in alcohol and serial dilutions were made with water. Inhibition and reversal studies on *L. leichmannii* 313 were conducted in the manner described previously with other inhibitors.[9]

Animal Assay.—The compound was assayed for androgenic activity by our standard method.[12] For antiandrogenic activity, we followed the same procedure but gave the rats, in addition to the test compound, 1 γ of testosterone daily, subcutaneously. In the chick assay, the compound was administered locally in addition to 1 γ of testosterone daily on the comb. The final assessment of antiandrogenicity was done by the method of Dorfman[13] where the comb is stimulated by a single intramuscular injection of 0.5 mg. of testosterone enanthate and the antagonist is applied daily to the comb.

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Ethyl N-Benzylleucinate Nitrogen Mustard[1]

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In the treatment of some animal tumors, the differences in the activities and toxicities of alkylating agents[2–4] which accompany variations in structure and configuration and the marked effectiveness of some agents, such as sarcolysin (I),[4] incorporating a nitrogen mustard alkylating group attached to an amino acid carrier, are well known. They suggest that the preparation of related amino acid nitrogen mustards may afford active agents which may be of greater use in cancer chemotherapy than those presently available and may also give additional insight into the exact mode of action of alkylating agents.[5]

As a contribution to the achievement of these goals ethyl N-(*p*-[bis(2-chloroethyl)amino]benzylidene)-DL-leucinate (II) was prepared by the condensation of *p*-[bis(2-chloroethyl)amino]benzaldehyde[6] and ethyl DL-leucinate. Reduction of the Schiff base II with hydrogen, using Adams catalyst, produced ethyl N-(*p*-[bis(2-chloroethyl)amino]benzyl)-DL-leucinate (III), isolated as the oxalate salt. Both II and III were

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