gave XI, m.p. 241–243°, λ_{max}^{MeOH} 260 mμ (log ε 4.20), $\lambda_{max}^{CHCl_3}$ 2.74, 2.8–2.95 (OH), 5.73, 8.08 (acetate), 5.77μ (20 C=O).

Anal. Calcd. for $C_{31}H_{36}F_2N_2O_5$: C, 67.07; H, 6.49. Found: C, 67.06; H, 6.66.

Acknowledgment.—The authors wish to thank Dr. L. H. Sarett and Dr. A. A. Patchett for many stimulating discussions. We are indebted to Mr. R. Boos and his collaborators for analyses, to Mr. E. MacMullan for ultraviolet spectra, and to Mr. R. Walker and Mr. N. Allen for infrared spectra.

Notes

Synthesis of 2′-(2,4-Difluorophenyl)-11β,17,21-trihydroxy-6,16α-dimethyl-20-oxopregna-4,6-dieno[3,2-*c*]pyrazole

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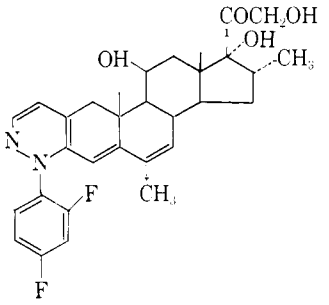
It has been shown that 2′-phenylpregn-4-eno[3,2-*c*]-pyrazoles derived from cortisol or other glucocorticoids are more potent antiinflammatory agents than the parent 3-keto-Δ^4-steroids.[1-4] A variety of compounds carrying substituents in the aromatic ring were also prepared and tested, but these were less active than the unsubstituted phenylpyrazole.[5] The only exception proved to be the *p*-fluorophenyl derivative[1,3] which was about 1.65 times as active as the phenylpyrazole and 6.5 times as active as the *p*-chlorophenylpyrazole[3] in the 16α-methylcortisol series. Since a small, electron-withdrawing group in the *para* position was thus activity enhancing, it appeared of interest to prepare the *o,p*-difluorophenylpyrazole (III). In the oral granuloma inhibition assay[6] III proved to be about 115[7] times as active as cortisol, whereas its *ortho*-unsubstituted analog[2] was 600 times as active as cortisol.



III

Experimental

2′-(2,4-Difluorophenyl)-11β-hydroxy-6,16α-dimethyl-17,20:20,21-bis(methylenedioxy)pregna-4,6-dieno[3,2-*c*]pyrazole (II).—To 500 mg. (1.09 mmoles) of 11β-hydroxy-2-hydroxymethylene-6,16α-dimethyl-17,20:20,21-bis(methylenedioxy)-pregna-4,6-dien-3-one (I),[2] in 5 ml. of glacial acetic acid was added 108 mg. (1.31 mmoles) of sodium acetate and 238 mg. (1.31 mmoles) of 2,4-difluorophenylhydrazine hydrochloride. The solution was stirred for 30 min. in an atmosphere of nitrogen and then was filtered to remove a small amount of insoluble material. Water was added and a flocculent precipitate formed which was extracted into methylene chloride. The methylene chloride layer was washed successively with a cold solution of dilute hydrochloric acid, water, a dilute solution of sodium hydroxide, water, and saturated sodium chloride solution, and was dried over magnesium sulfate. The solution was concentrated to dryness to yield 627 mg. of a light yellow foam. The crude product was dissolved in a minimum amount of benzene and chromatographed on 17.5 g. of neutral alumina. Benzene–methylene chloride mixtures (9:1 and 7:3) eluted 420 mg. of II. Crystallization from acetone–hexane afforded 100 mg. of crystalline II, m.p. 215–217°, $\lambda\lambda_{max}^{MeOH}$ 312 mμ (log ε 4.31), 269 (4.15). The ultraviolet spectrum is in accord with the assigned structure.[1-3] An analytical sample prepared by repeated recrystallization from acetone–hexane melted at 216–217°.

Anal. Calcd. for $C_{32}H_{36}F_2N_2O_5$: C, 67.75; H, 6.41. Found: C, 67.56; H, 6.28.

2′-(2,4-Difluorophenyl)-11β,17,21-trihydroxy-6,16α-dimethyl-20-oxopregna-4,6-dieno[3,2-*c*]pyrazole (III).—A mixture of 320 mg. of II and 25 ml. of 60% aqueous formic acid was heated under nitrogen on a steam bath for 35 min. and was concentrated *in vacuo* to dryness. Addition of 3 ml. of water followed by vigorous agitation of the mixture gave 268 mg. of a noncrystalline solid. The crude product was dissolved in 7 ml. of methanol, 0.07 ml. of a 2.3 *N* solution of sodium methoxide was added, and the solution was stirred in an atmosphere of nitrogen for 10 min. The base was neutralized with glacial acetic acid, and the solution was concentrated to dryness. The residue was dissolved in methylene chloride, washed with water, and dried over magnesium sulfate. After concentrating the solution to dryness, the residue was dissolved in hot acetone and hexane was added to afford crystalline III. Recrystallization from acetone gave an analytical sample, m.p. 224–226.5°, $\lambda\lambda_{max}^{MeOH}$ 312.5 mμ (log ε 4.30), 270 (4.14).

Anal. Calcd. for $C_{30}H_{34}F_2N_2O_4 \cdot 0.5C_3H_6O$: C, 68.35; H, 6.72. Found: C, 68.30; H, 6.58.

(1) R. Hirschmann, N. G. Steinberg, P. Buchschacher, J. H. Fried, G. J. Kent, M. Tishler, and S. L. Steelman, *J. Am. Chem. Soc.*, **85**, 120 (1963).

(2) J. H. Fried, H. Mrozik, G. E. Arth, T. S. Bry, N. G. Steinberg, M. Tishler, R. Hirschmann, and S. L. Steelman, *ibid.*, **85**, 236 (1963).

(3) R. Hirschmann, P. Buchschacher, N. G. Steinberg, J. H. Fried, R. Ellis, G. J. Kent, and M. Tishler, *ibid.*, **86**, 1520 (1964).

(4) R. Hirschmann, N. G. Steinberg, E. Schoenewaldt, W. J. Paleveda, and M. Tishler, *J. Med. Chem.*, **7**, 352 (1964).

(5) R. Hirschmann and S. L. Steelman, unpublished observations.

(6) S. L. Steelman and F. R. Morgan, "Inflammation and Diseases of Connective Tissues," L. C. Mills and J. H. Moyer, Ed., W. B. Saunders Co., Philadelphia, Pa., 1961, p. 349.

(7) We are greatly indebted to Dr. S. L. Steelman for permission to report this result.

17-Substituted 3β-Hydroxy-4-pregnen-20-ones

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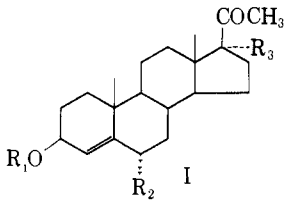
A number of communications have appeared on the synthesis of 3β-hydroxy-Δ^4-pregnenes and -androstenes,[1] and some compounds of this class have shown physiological activity. For example, 3β-hydroxy-4-pregnen-20-one[1a] and its 6α-methyl derivative[1f] are

(1) (a) M. Gut, *J. Org. Chem.*, **21**, 1327 (1956); (b) S. Bernstein, S. M. Stolar, and M. Heller, *ibid.*, **22**, 472 (1957); (c) F. Sondheimer and Y. Klibansky, *Tetrahedron*, **5**, 15 (1959); (d) J. S. Baran, *J. Med. Chem.*, **6**, 329 (1963); (e) F. B. Colton and P. Klimstra, *Excerpta Med.*, Intern. Congr. Ser., No. 51, Intern. Congr. on Hormonal Steroids, Milan, 1962, p. 57; (f) B. Löken, M. Uskoković, M. Hagopian, R. I. Dorfman, and M. Gut, *Steroids*, **2**, 81 (1963).

TABLE 1
3β-HYDROXY-4-PREGNEN-20-ONES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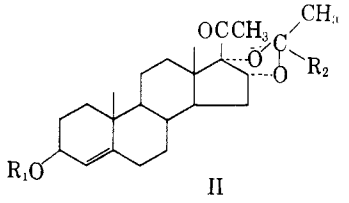
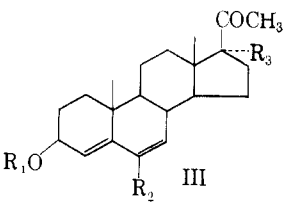

II


III

No.	R_1	R_2	R_3	Crystn. solvent	M.p., °C.	$[\alpha]D$	Formula	% carbon Calcd.	% carbon Found	% hydrogen Calcd.	% hydrogen Found
Ia	H	H	AcO	MeOH–H_2O	198–200.5	+24.5	$C_{23}H_{34}O_4$	73.75	73.98	9.15	9.39
Ib	Ac	H	AcO	MeOH–H_2O	165–167.5	+1.1	$C_{25}H_{36}O_5$	72.09	72.36	8.71	8.74
Ic	H	CH_3	AcO	Et_2O–C_6H_{14}[a]	182–185	+21	$C_{24}H_{36}O_4$	74.57	74.25	8.87	9.22
Id	Ac	CH_3	AcO	MeOH	164–166	−13	$C_{26}H_{38}O_5$	72.52	72.26	8.90	8.60
Ie	H	F	AcO	Me_2CO–C_6H_{12}	147–147.5	+27	$C_{23}H_{33}FO_4$	70.39	70.64	8.47	8.24
If	Ac	F	AcO	MeOH	206 dec.	−8.1	$C_{25}H_{35}FO_5$	69.10	69.61	8.12	8.10
Ig	H	H	CH_3	MeOH[a]	186–191	+56	$C_{22}H_{34}O_2$	79.97	80.19	10.37	10.56
IIa	H	CH_3		MeOH–H_2O[c]	167–169	+85	$C_{21}H_{36}O_4$	71.20	73.94	9.34	9.15
IIb	Ac	CH_3		CH_2Cl_2–MeOH	195–196.5	+43	$C_{26}H_{38}O_5$	72.51	72.81	8.89	9.03
IIc	H	C_6H_5		Et_2O–C_6H_{14}[c]	132–140	+25	$C_{29}H_{38}O_4$	77.29	77.21	8.50	8.41
IId	Ac	C_6H_5		CH_2Cl_2–MeOH	165–167	−12	$C_{31}H_{40}O_5$	75.58	75.50	8.19	7.95
IIIa	H	H	AcO	Me_2CO–C_6H_{14}[b]	178–180	−61	$C_{22}H_{32}O_4$	74.16	74.04	8.66	8.53
IIIb	Ac	H	AcO	Me_2CO–C_6H_{14}	193–194	−94	$C_{25}H_{34}O_5$	72.43	72.30	8.27	8.18
IIIc	H	CH_3	CH_3	Me_2CO–C_6H_{14}[b]	193–194	−16	$C_{23}H_{34}O_2$	80.65	80.67	10.01	9.77

[a] Chromatographed on Florisil. [b] Chromatographed on alumina. [c] Purified *via* the acetate which was hydrolyzed with potassium carbonate in aqueous methanol (1 hr. reflux).

reported to have progestational activity of the same order of magnitude as that of the corresponding progesterones.[2] The possibility that 3-hydroxy compounds of this type are interconvertible *in vivo* with the corresponding Δ^4-3-ketones is raised by the recent isolation of 3α,17α-dihydroxy-4-pregnen-20-one after perfusion of 17α-hydroxyprogesterone through bovine adrenals and ovaries.[3]

As part of a study of derivatives of 17-substituted progesterones, we have prepared and tested a series of 3β-hydroxy-4-pregnen-20-ones with various substituents at C-17. The required compounds were prepared by reduction of the corresponding progesterones with lithium aluminum tri-*t*-butoxyhydride. In all cases, preferential reduction of the 3-keto group occurred, as evidenced by infrared spectra, giving the 3β-hydroxy-4-pregnen-20-ones listed in Table I. The configuration of the hydroxyl group follows from the fact that 4-cholesten-3-one has been shown to yield only the 3β-hydroxy isomer on reduction with lithium aluminum tri-*t*-butoxyhydride.[4]

After most of this work had been completed, it was found that, at least in several cases, the same products could be obtained in good yield by reduction with a limited amount of sodium borohydride in methanol. Since unsubstituted 20-ketones are reduced in preference to Δ^4-3-ketones by sodium borohydride in methanol,[5,6] the success of the borohydride reductions is presumably due to steric hindrance provided by the 17-substituent.

Biological Activity.—The progestational activity of some of the 3β-hydroxy-4-pregnen-20-ones in the McPhail modification of the Clauberg test[7] relative to progesterone (subcutaneous) and 17α-ethynyl-19-nortestosterone (oral) is shown in Table II. In general, the 3β-hydroxy compounds had activity of the same order of magnitude as the corresponding Δ^4-3-ketones.[8]

Compounds Ic and IIIc maintained pregnancy in ovariectomized rats, but Ia, Ie, and IIa did not. Compound Ic suppressed gonadotrophin activity in parabiotic rats and caused masculinization of female rat fetuses; its behavior was thus similar to that of the corresponding ketone, 6α-methyl-17-acetoxyprogesterone.

Experimental[9]

Reduction with Lithium Aluminum Tri-*t*-butoxyhydride.—A solution of 1.50 g. of 6α-methyl-17-acetoxyprogesterone[10] in 39 ml. of tetrahydrofuran was added dropwise to 2.97 g. of lithium aluminum tri-*t*-butoxyhydride in 12 ml. of tetrahydrofuran. After stirring for 3 hr. at room temperature, 12 ml. of acetone was added to decompose excess hydride and stirring was continued for an additional 30 min. Sufficient saturated sodium sulfate solution was then added to precipitate the inorganic salts, the mixture was filtered, and the filtrate was concentrated *in vacuo*. Ether extraction gave a crude product which was chromatographed on Florisil. The material eluted with benzene containing 5% ether was crystallized from ether–hexane yielding 0.52 g. of 17-acetoxy-3β-hydroxy-6α-methyl-4-pregnen-20-one (Ic), m.p. 180–185°. The 3,17-diacetate (Id) was made by acetylation overnight at room temperature in acetic anhydride–pyridine.

The other compounds listed in Table I were prepared by essentially the same procedure.

Reduction with Sodium Borohydride.—16,17-Isopropylidenedioxyprogesterone[11] (200 mg., 0.52 mmole) was added to an

(2) After completion of most of this work, it was reported that 17-acetoxy-3β-hydroxy-4-pregnen-20-one has about the same activity as the corresponding 3-ketone (R. Elton and V. A. Drill, *Excerpta Med.*, Intern. Congr. Ser., No. 51, Intern. Congr. on Hormonal Steroids, Milan, 1962, p. 12).

(3) H. Levy, T. Saito, and S. Takeyama, *Biochim. Biophys. Acta*, **69**, 198 (1963).

(4) O. H. Wheeler and J. L. Mateos, *Can. J. Chem.*, **36**, 1431 (1958).

(5) J. K. Norymberski and G. F. Woods, *J. Chem. Soc.*, 3426 (1955).

(6) D. Kupfer, *Tetrahedron*, **15**, 193 (1961), has reported the isolation of 3β-hydroxy-4-pregnen-20-one from the reduction of progesterone with sodium borohydride in 2-propanol.

(7) M. K. McPhail, *J. Physiol.*, **83**, 145 (1934).

(8) Since the data for the ketones corresponding to Ie, IIa, and IIc were taken from the literature, the comparison in these cases between alcohols and ketones is only very approximate.)

(9) Melting points were determined in a Herschberg-type apparatus and are corrected. Rotations were determined in chloroform solution at a concentration of about 1%. The alumina used for chromatography was Woelm neutral alumina, Activity III.

(10) J. C. Babcock, E. S. Gutsell, M. E. Herr, J. A. Hogg, J. C. Stucki, L. E. Barnes, and W. E. Dulin, *J. Am. Chem. Soc.*, **80**, 2904 (1958).

(11) G. Cooley, B. Ellis, F. Hartley, and V. Petrow, *J. Chem. Soc.*, 4373 (1955).

TABLE II

RELATIVE PROGESTATIONAL ACTIVITY (CLAUBERG ASSAY)

Progesterone	S.c.	Oral	Corresponding Δ⁴-3-ketone S.c.	Corresponding Δ⁴-3-ketone Oral
Progesterone	1			
17α-Ethynyl-19-nortestosterone		1		
Ia	2	0.4		0.1[a]
Ib	2	0.4		
Ic	10	10	20[a]	10[a]
Ie	1	0.4		1[b]
IIa	1	0.05	1–2[c]	0.15[c]
IIc	1	0.4	1–2[c]	1–2[c]
IIIc	10	<10	4[a,d]	10[a,d]

[a] Determined in this laboratory. [b] A. Bowers, L. C. Ibanez, and H. J. Ringold, *J. Am. Chem. Soc.*, **81**, 5991 (1959). [c] J. Fried, E. F. Sabo, P. Grabowich, L. J. Lerner, W. B. Kessler, D. M. Brennan, and A. Borman, *Chem. Ind.* (London), 465 (1961). [d] R. Deghenghi and R. Gaudry, *J. Am. Chem. Soc.*, **83**, 4668 (1961).

ice-cooled solution of 39 mg. (1.04 mmoles) of sodium borohydride in 5 ml. of dry methanol. After stirring for 1.5 hr., 0.1 ml. of acetic acid was added and the solution was evaporated to dryness *in vacuo*. Extraction with chloroform gave a solid product which was identified as almost pure 3β-hydroxy-16α, 17α-isopropylidenedioxy-4-pregnen-20-one (IIa) by infrared spectral and thin layer chromatographic comparison with the compound obtained by reduction of the same starting material with lithium aluminum tri-*t*-butoxyhydride.

When 17-acetoxyprogesterone was reduced with sodium borohydride under the same conditions, the crude product consisted of the corresponding 3β-hydroxy compound (Ia) contaminated with a small amount of starting material.

Acknowledgments.—We are indebted to Dr. G. Papineau-Couture and Mrs. J. Jachner for infrared spectra, to Mr. W. J. Turnbull and associates for the microanalyses, and to D. Stoltzenburg and R. Guthrie for technical assistance.

A Nitrogen Mustard Derivative of 6-Mercaptopurine[1]

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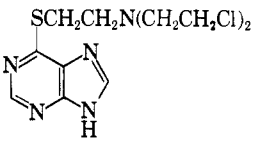
The carcinostatic effects of a variety of nitrogen mustards[2a] and of 6-mercaptopurine[2b] (6-MP) made it of interest to study a combination of the two in which the purine was the carrier for the alkylating group. Such a derivative, 6-[[2-[bis(2-chloroethyl)amino]-ethyl]thio]purine (I) was prepared by the alkylation

TABLE I

EFFECT OF THE NITROGEN MUSTARD DERIVATIVE OF 6-MERCAPTOPURINE (I) AND OF 6-MP ON MOUSE, RAT, AND HAMSTER TUMORS[a]

Tumor	I 2.5 mg./kg./day	HN2 0.5 mg./kg./day	6MP 30 mg./kg./day
Ehrlich carcinoma (ascitic)	+++	+++	++
Sarcoma 180 (ascitic)	+++	+++	+++
Mecca lymphosarcoma	+	±	−
Friend virus leukemia	+	+	++
Ehrlich carcinoma	±	±	+
Carcinoma 1025	±	+	++
Ridgway osteogenic sarcoma	±	−	++
Wagner osteogenic sarcoma	±	−	+
Glioma 26	±	−	++
Sarcoma 180	−	±	+++
Adenocarcinoma E 0771	−	±	++
Lewis lung carcinoma	−	−	−
Harding–Passey melanoma	−	−	−
Flexner–Jobling rat carcinoma	+++	+	++
Jensen rat sarcoma	++	+	+
Walker carcinosarcoma 256	+	+	±
Crabb hamster sarcoma	±	+	+

[a] −, no effect; ±, slight inhibition; +, moderate inhibition; ++, marked inhibition; +++, complete inhibition or regressions of tumors.



I

procedure of Johnston, *et al.*,[3] from tris(2-chloroethyl)-amine and 6-mercaptopurine in N,N-dimethylformamide containing triethylamine. It was isolated as a dihydrochloride and half of the halogen present was found to be ionic. Its ultraviolet absorption spectrum corresponded to that expected of a 6-alkylthiol derivative. The possibility of ring alkylation is eliminated since only hypoxanthine was formed by oxidative hydrolysis in dilute hydrogen peroxide at room temperature.

Compound I was tested at, or near, the maximum tolerated dose for carcinostatic activities against seventeen tumors. Similar tests with methyl bis(2-chloroethyl)amine (HN2) and with 6-MP are recorded in Table I.

The maximum tolerated dose of the compound was comparable to that of HN2 and was approximately one-tenth that of 6-MP. From the results (Table I) on the spectrum of tumors, it is apparent that the antitumor activity of I paralleled that of HN2 more closely than it did that of 6-MP. It was definitely less effective than 6-MP[4] on the mouse tumors.

Experimental

6-[[2-[Bis(2-chloroethyl)amino]ethyl]thio]purine (I) Dihydrochloride.—To a solution of 0.5 g. of 6-mercaptopurine in 15 ml. of dimethylformamide, 2.3 g. of tris(2-chloroethyl)amine hydro-

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (CA-03190-07), Cancer Chemotherapy National Service Center Contract SA-43-ph-2445, and from the Atomic Energy Commission (Contract No. AT(30-1)-910).

(2) (a) E. Hirschberg, *Cancer Res.*, **23**, 548 (1963); (b) *ibid.*, **23**, 593 (1963).

(3) T. P. Johnston, L. B. Holm, and J. A. Montgomery, *J. Am. Chem. Soc.*, **80**, 6265 (1958).

(4) K. Sugiura, in "Progress in Experimental Tumor Research," Vol. II, F. Homburger, Ed., S. Karger, New York, N. Y., 1961, p. 332.