The methoxime **10**, prepared by the reaction of **6** with methoxamine, was also rapidly dehydrogenated with DDQ yielding the 6-methoximinomethyl dienone **11**.

Biology.§ Table I lists the intramuscular progestational activities of **3**, **4**, **11**, and several of the intermediates.

Experimental Section#

3-Ethoxy-6-formyl-16-methylene-17α-hydroxy-3,5-pregnadien-20-one 17-Acetate (6). A soln of $POCl_3$ (9.7 ml) in DMF (81.5 ml) was added dropwise to a suspension of 3-ethoxy-16-methylene-17α-hydroxy-3,5-pregnadien-20-one 17-acetate (**5**) (16.3 g) in DMF (163 ml) at 20°. The reaction mixt was stirred for 2.5 hr, then added to H_2O (4 l.) contg KOAc (100 g). After stirring for 1 hr the ppt was collected by filtration. Crystn from EtOH afforded 13 g (75%) of **6**: mp 170–174°; [α]D −255° ($CHCl_3$); λ_{max} 219 mμ (ε 11,300), 325 (15,300). *Anal.* ($C_{27}H_{36}O_5$) C, H.

6-Formyl-16-methylene-17α-hydroxy-4,6-pregnadiene-3,20-dione 17-Acetate (3). A soln of 6 (353 mg) in 95% aqueous Me_2CO (7.5 ml) was stirred with DDQ (187 mg) at 20° for 105 min. The reaction mixt was passed through a short column of neutral alumina (Woelm, act. I). Elution with $CHCl_3$–MeOH (1:1) followed by crystn from Me_2CO–C_6H_{14} gave 152 mg (45%) of **3**: mp 210–212°; [α]D −152° ($CHCl_3$); λ_{max} 278 mμ (ε 22,000); ν_{max} 1725, 1700, 1685, 1645 cm^{-1}; nmr, δ 5.43 and 5.62 (C_{16}=CH_2), 9.63 (C_6CHO) ppm. *Anal.* ($C_{25}H_{30}O_5$) C, H.

3-Ethoxy-6-oximinomethyl-16-methylene-17α-hydroxy-3,5-pregnadien-20-one 17-acetate (7) was prepd from 6 by the method described in ref 6 in 63% yield after crystn from EtOH–H_2O: mp 135–140°; [α]D −286°; λ_{max} 296 mμ (ε 21,000). *Anal.* ($C_{27}H_{37}O_5N \cdot 0.5H_2O$) C, H, N.

3-Ethoxy-6-cyano-16-methylene-17α-hydroxy-3,5-pregnadien-20-one 17-acetate (8) was prepd from 7 by the method described in ref 6 in 66% yield after crystn from EtOH–*i*-Pr_2O: mp 120–125°; [α]D −250°; λ_{max} 284 mμ (ε 19,700). *Anal.* ($C_{27}H_{35}O_4N$) C, H, N.

6-Oximinomethyl-16-methylene-17α-hydroxy-4,6-pregnadiene-3,20-dione 17-Acetate (9). A soln of 7 (3.48 g) in 95% aqueous Me_2CO (80 ml) was stirred with DDQ (3 g) at 20° for 45 min. The reaction mixt was passed through a column of neutral alumina (Woelm, act. I). Elution with $CHCl_3$ gave 2.21 g (68.6%) of homogeneous **9**. A small part was crystd from EtOAc affording an EtOAc solvate: mp 140–145°; [α]D −103°; λ_{max} 245 mμ (ε 11,620) 287 (16,000). *Anal.* ($C_{25}H_{31}NO_5 \cdot 0.5$EtOAc) C, H, N.

6-Cyano-16-methylene-17α-hydroxy-4,6-pregnadiene-3,20-dione 17-Acetate (4). A soln of 9 (488 mg) in pyridine (5 ml) was stirred with $POCl_3$ (2.2 ml) under N_2 for 2 hr. The soln was added to H_2O, and the ppt collected and dried. Crystn from EtOAc gave (in 2 crops) 329 mg (70%) of **4**: mp 221–223°; [α]D −141°; λ_{max} 275 mμ (ε 23,800); ν_{max} 2237, 1750, 1721, 1670, and 1638 cm^{-1}; nmr, δ 0.80 ($C_{13}CH_3$), 1.15 ($C_{10}CH_3$), 2.04 ($C_{17}OCOCH_3$), 2.17 ($C_{20}CH_3$), 5.52 and 5.67 (C_{16}=CH_2), 6.24 (C_4H), and 6.89 (C_7H) ppm. *Anal.* ($C_{25}H_{29}O_4N$) C, H, N.

3-Ethoxy-6-methoximinomethyl-16-methylene-17α-hydroxy-3,5-pregnadien-20-one 17-Acetate (10). A soln of 6 (500 mg), methoxamine·HCl (95 mg), and NaOAc (186 mg) in EtOH (10 ml) and H_2O (2 ml) was heated at reflux for 30 min, then allowed to stand at 20° for 16 hr. The soln was added to H_2O, extd with CH_2Cl_2, and dried. The solvent was evapd *in vacuo* and the residue crystd from MeOH–H_2O affording 423 mg (79%) of **10**: mp 95–98°; [α]D −271°; λ_{max} 222 mμ (ε 9900), 302 (21,400). *Anal.* ($C_{28}H_{39}O_5N$) C, H, N.

6-Methoximinomethyl-16-methylene-17α-hydroxy-4,6-pregnadiene-3,20-dione 17-Acetate (11). A soln of 10 (100 mg) in 95% aqueous Me_2CO (10 ml) was stirred with DDQ (100 mg) at 20° for 30 min. Most of the solvent was removed *in vacuo* and the residue chromatogd over neutral alumina (Woelm, act. I). Elution with $CHCl_3$–EtOAc (1:1) afforded after crystn from Me_2CO–H_2O 52 mg (55.7%) of **11**: mp 185–191°; [α]D −88°; λ_{max} 260 mμ (ε 15,000), 283 (16,500); nmr, δ 3.90 (=$NOCH_3$), 5.49 and 5.64 (C_{16}=CH_2), 6.27 (C_4H), 6.42 (C_7H), and 7.88 (C_6CH=$NOCH_3$) ppm. *Anal.* ($C_{26}H_{33}O_5N$) C, H, N.

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§ We are indebted to Dr. R. O. Neri and associates, Physiology and Biochemistry Department, Schering Corp., for carrying out the biological screening.

Melting points are uncorrected. Rotations are in dioxane at 25° at about 1% concentration, uv spectra are of MeOH solutions, and ir spectra are in Nujol unless otherwise stated. The nmr spectra were measured on a Varian A 60-A spectrometer in $CDCl_3$ (Me_4Si). Solns were dried over anhyd Na_2SO_4. Analyses were determined by the Physical Organic Department of the Schering Corp. Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

Antiinflammatory Activities of 17,21-Methyl Ortho Esters, 17-Mono- and 17,21-Diesters of 6α,9α-Difluorocorticosteroids

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Corticosteroid 17,21-alkyl ortho esters were prepared some years ago in our laboratory.[1] Their acid-catalyzed hydrolysis gave rise to corticosteroid 17-monoesters, which were conventionally acylated to the corresponding 17,21-diesters.[2,3] Several derivatives of the above 3 classes, obtained from various parent corticoids, proved to display extremely interesting antiinflammatory activity, mainly after local treatment.[4-8]

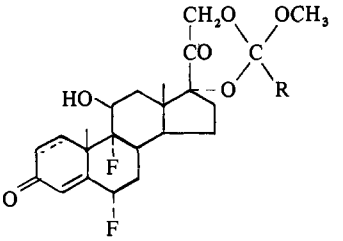
This paper reports the synthesis and some biological properties of 17,21-alkyl ortho esters, 17-monoesters, and 17,21-diesters of 6α,9α-difluorocortisol and 6α,9α-difluoroprednisolone, known potent antiinflammatory steroids.[9,10]

Chemistry. 17,21-Alkyl ortho esters were prepared from 6α,9α-difluorocortisol and 6α,9α-difluoroprednisolone by exchange reaction with trimethyl ortho esters according to the already published procedure.[1] Hydrolysis of alkyl ortho esters was performed in buffered solution at pH near 5. In these conditions both direct hydrolysis to 21-ester and acyl migration 17-O → 21-O were minimized and 17-monoesters were obtained in optimum yield.[11,12]

Acylation of 17-monoesters, although performed in conventional manner, required a careful control of the temperature below 0° in order to avoid extensive formation of triesters, since the presence of an acyloxy group in 17α position was found to significantly enhance reactivity of 11β-OH.†

† This matter will be discussed in a forthcoming paper.

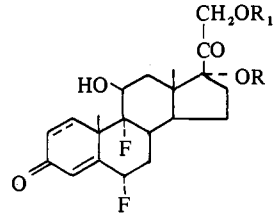
Table I



No.	Δ[a]	R	Yield,[b] %	Recrystn solvent	Mp, °C[d]	[α]D[d]	Formula[c]
1	1,4	CH_3	47	Et_2O-CH_2Cl_2	222-225	+71	$C_{24}H_{30}F_2O_6$
2	1,4	C_2H_5	59	Et_2O-petr ether	215-219	+64.5	$C_{25}H_{32}F_2O_6$
3	4	C_3H_7	63	MeOH	197-201	+84	$C_{26}H_{36}F_2O_6$
4	1,4	C_3H_7	65	CH_2Cl_2-MeOH	194-198	+59	$C_{26}H_{34}F_2O_6$
5	1,4	$(CH_3)_2CH$	54	CH_2Cl_2-MeOH	230-232	+55.5	$C_{26}H_{34}F_2O_6$
6	4	C_4H_9	60	Et_2O-petr ether	179-183	+70.5	$C_{27}H_{38}F_2O_6$
7	1,4	C_4H_9	60	Et_2O-CH_2Cl_2	182-184[e]	+54.5	$C_{27}H_{36}F_2O_6$

[a]Ring A unsaturation. [b]Yield is of analytically pure material. [c]All compds were analyzed for C, H. [d]Relevant to samples containing about 90% of the more polar isomer. [e]With softening at 167°.

Table II



No.	Δ[a]	R	R_1	Yield,[b] %	Recrystn solvent	Mp, °C	[α]D	Formula[c]
8	1,4	CH_3CO	H	77	Me_2CO-Et_2O	218-222	+11	$C_{23}H_{28}F_2O_6$
9	1,4	CH_3CH_2CO	H	74	Me_2CO-Et_2O	212-215	+9	$C_{24}H_{30}F_2O_6$
10	1,4	$CH_3(CH_2)_2CO$	H	79	Me_2CO-Et_2O	193-196	+7	$C_{25}H_{32}F_2O_6$
11	1,4	$(CH_3)_2CHCO$	H	73	Me_2CI-Et_2O	219-221	+7.5	$C_{25}H_{32}F_2O_6$
12	4	$CH_3(CH_2)_3CO$	H	58	Me_2CO-Et_2O	186-190	+31.5	$C_{26}H_{36}F_2O_6$
13	1,4	$CH_3(CH_2)_3CO$	H	75	Me_2CO-Et_2O	167-169	+6	$C_{26}H_{34}F_2O_6$
14	1,4	CH_3CO	CH_3CO	94	CH_2Cl_2-Et_2O	280-283	+31	$C_{25}H_{30}F_2O_7$
15	1,4	CH_3CO	$CH_3(CH_2)_2CO$	84	CH_2Cl_2-Et_2O	246-248	+35.5	$C_{27}H_{34}F_2O_7$
16	1,4	CH_3CO	$(CH_3)_2CHCO$	89	CH_2Cl_2-Et_2O	241-243	+34.5	$C_{27}H_{34}F_2O_7$
17	1,4	CH_3CH_2CO	CH_3CO	87	CH_2Cl_2-Et_2O	229-232	+30.5	$C_{26}H_{32}F_2O_7$
18	1,4	CH_3CH_2CO	CH_3CH_2CO	87	CH_2Cl_2-Et_2O	227-231	+33	$C_{27}H_{34}F_2O_7$
19	1,4	CH_3CH_2CO	$CH_3(CH_2)_2CO$	90	Et_2O-petr ether	213-215	+36	$C_{28}H_{36}F_2O_7$
20	1,4	CH_3CH_2CO	$(CH_3)_2CHCO$	85	MeOH	180-182	+32.5	$C_{28}H_{36}F_2O_7$
21	1,4	CH_3CH_2CO	$(CH_3)_3CCO$	69	CH_2Cl_2-ether	243-245	+34	$C_{29}H_{38}F_2O_7$
22	1,4	$CH_3(CH_2)_2CO$	CH_3CO	45	EtOH	192-194[d]	+30	$C_{27}H_{34}F_2O_7$
23	1,4	$CH_3(CH_2)_2CO$	CH_3CH_2CO	91	MeOH-ether	198-201	+33	$C_{28}H_{36}F_2O_7$
24	1,4	$CH_3(CH_2)_2CO$	$CH_3(CH_2)_2CO$	90	Et_2O-petr ether	215-217	+33.5	$C_{29}H_{38}F_2O_7$
25	1,4	$CH_3(CH_2)_2CO$	$(CH_3)_2CHCO$	95	Et_2O-petr ether	223-225	+33	$C_{29}H_{38}F_2O_7$
26	1,4	$(CH_3)_2CHCO$	CH_3CO	80	CH_2Cl_2-Et_2O	186-190	+29.5	$C_{27}H_{34}F_2O_7$
27	1,4	$(CH_3)_2CHCO$	CH_3CH_2CO	85	CH_2Cl_2-Et_2O	208-212	+32	$C_{28}H_{36}F_2O_7$
28	1,4	$(CH_3)_2CHCO$	$(CH_3)_2CHCO$	82	CH_2Cl_2-Et_2O	181-185	+34	$C_{29}H_{38}F_2O_7$
29	4	$CH_3(CH_2)_3CO$	CH_3CO	88	Et_2O-petr ether	155-156.5	+49	$C_{28}H_{38}F_2O_7$
30	1,4	$CH_3(CH_2)_3CO$	CH_3CO	86	CH_2Cl_2-Et_2O	207-209	+29	$C_{28}H_{36}F_2O_7$

[a-c]See Table I. [d]Samples recrystd from MeOH or Et_2O-petr ether (mp 174-192°) consisted of two polymorphic forms. (Personal communication by H. A. Kessler, Warner-Lambert Research Institute, Morris Plains, N. J.).

The yields, melting points, specific optical rotations, and analytical data of the compounds are given in Tables I and II.

Biology and Evaluation. The compounds were assayed for their antiinflammatory activity by the granuloma pouch test according to Selye.[13] Rats were injected into pouch on day 5 and autopsied on day 11. Several compounds proved to be extremely active in reducing exudate. In particular ortho esters **4**, **5**, **6**, and **7**, 17-monoesters **12** and **13**, and diester **26**, were at least 1000 times as active as cortisol, since their ED_{50} was lower than 0.01 μmoles *vs.* about 10 μmoles for cortisol.

The compounds were also assayed in the vasoconstriction test in humans, reported to give results which parallel satisfactorily the clinical antiinflammatory activity.[14] The test was performed on volunteers according to the modification described by Falconi and Rossi.[15,16] The results are summarized in Table III. Most tested steroids appeared to be many times more active than free 6α,9α-difluoroprednisolone. Several compounds were more active than β-methasone 17-valerate.[4] The most potent derivatives were 17,21-diesters, particularly **17**, **18**, **19**, **20**, **22**,‡ **23**, and **25**.

Where a comparison was made, $\Delta^{1,4}$ compounds (**4**, **7**, **13**,

‡For other biological assays on **22** see ref 17.

Table III. Activity of 6α,9α-Difluoroprednisolone Derivatives in the Vasoconstriction Test in Humans[a]

Compd	Relative potency[c]
Betamethasone 17-valerate[b]	1
6α,9α-Difluoroprednisolone	<0.1
3	1
4	1
6	1
12	1
13	1
14	1
16	2
17	3
18	3
19	3.5
20	3
21	2
22	3.5
23	3
24	1.5
25	3
26	2
27	2.5
28	2
29	1
30	2

[a]Each compd was texted on 24 subjects at least at three dose levels (0.015–0.18 mcg). [b]0.1 μg induced 50% of maximum blanching score. [c]Compds of Tables I and II not listed here displayed potency <1.

30) exhibited [*vs.* the corresponding Δ^4 compounds (3, 6, 12, 29, respectively)] either no advantage or an advantage by far lower than that reported for 6α,9α-difluoroprednisolone 21-acetate systemically given.[10]

Experimental Section§

17,21-Methyl Ortho Esters (1–7). The procedure of Gardi, *et al.*,[1] was used. Isomeric mixts were obtd in almost quant yield which could be used directly in the subsequent step. Analytical samples of Table I were obtd by chromatog on Al_2O_3 and crystn. This processing enhanced the content of the more polar isomer from 50% to about 90% (tlc evidence only). No attempt was made to isolate pure isomers.

17-Monoesters (8–13). The following modification of the original procedure of Gardi, *et al.*,[2,3] was used. To a soln of the proper 17,21-methyl ortho ester (1 g) in MeOH (20 ml), NaOAc buffer (8 ml), prepd by mixing AcOH, 0.1 *N* soln (90 ml), and NaOAc, 0.1 *M* soln (10 ml), was added so to obtain a pH near 5. The reaction mixt was refluxed for 1 hr, concd under reduced pressure, and worked up in the usual way.

17,21-Diesters (14–30). The relevant 17-monoesters were acylated by treatment with the proper anhydride in pyridine at about −5° overnight. Products were isolated as usual and crystd.

Acknowledgment. The authors are indebted to Dr. C. Pedrali for the spectral determinations. We wish to thank Mr. G. Villa for chemical assistance and Mr. P. Beretta for assistance in the biological evaluations.

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§Melting points were taken in a capillary apparatus and are uncorrected. Optical rotations were detd in dioxane at 24° at a concn of about 0.5%. Uv were detd in 95% EtOH, and ir in Nujol mull. Absorption bands of these spectra were as expected. Tlc of analytical samples were done on silica gel GF with C_6H_6–Me_2CO 8:2 (1–7, 14–30) and 6:4 (8–13). Compounds 1–7 appeared as couples of closely moving spots, compounds 8–30 were homogeneous on tlc. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

Pyrido[3,2-*g*]pteridines. 1. Chemistry and Growth-Inhibitory Activity of Some 1*H*,3*H*-2,4-Dioxopyrido[3,2-*g*]pteridines (9-Azaalloxazines)†

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Recently we have been engaged in the synthesis and biological evaluation of some aza analogs of riboflavine, including 3*H*,10*H*-2,4-dioxo-7,8-dimethyl-10-[D(−)-ribityl]-pyrido[3,2-*g*]pteridine ("8-azariboflavine").‡ In connection with this program, we have also prepared some closely related 1*H*,3*H*-2,4-dioxopyrido[3,2-*g*]pteridines (9-azaalloxazines)§ for evaluation of their tumor growth-inhibitory action in selected *in vitro* and *in vivo* bioassay systems and for study of the chemistry of the azaalloxazine ring system. Except for one paper,[2] which reported the synthesis of 9-azaalloxazine (**1**) and 7-chloro-9-azaalloxazine (**2**) without spectral data and, in the case of **2**, with incomplete analytical data, nothing is reported in the literature on the chemistry and biology of this type of compound.

The compounds listed in Table I were obtained when the appropriately substituted diaminopyridine was condensed with alloxan monohydrate in glacial AcOH at room temp in the presence of $B(OH)_3$. With the exception of 2,3-diaminopyridine, which was obtained from a commercial source, the

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‡A brief account of this work has appeared.[1]

§The Chemical Abstracts numbering sequence for the pyrido[3,2-*g*]pteridine ring system is used in this report. The convenient expression "9-azaalloxazine" for 1*H*,3*H*-2,4-dioxopyrido[3,2-*g*]-pteridine derives from the common use of "alloxazine" for the benzo analog, 1*H*,3*H*-2,4-dioxobenzo[*g*]pteridine.