17α-ALKYNYL-11β,17-DIHYDROXYANDROSTANE DERIVATIVES : A NEW CLASS OF POTENT GLUCOCORTICOIDS.

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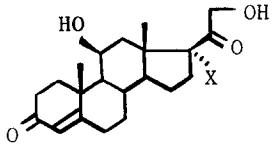
ABSTRACT

Replacement of the characteristic dihydroxyacetone side chain of corticoids by a 17α-alkynyl-17β-hydroxy moiety was investigated. It was found that, in particular, the 17α-propynyl substitution is favorable for high local anti-inflammatory activity with reduced systemic effects. Moreover, these compounds were found to be devoid of any affinity for the aldosterone receptor, and may thus be considered as pure glucocorticoids.

INTRODUCTION

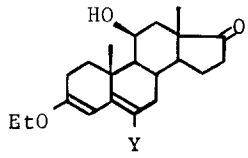
Natural corticosteroids possess the characteristic 17α,21-dihydroxy-20-keto substitution pattern of their pregnane side chain as exemplified by cortisol Ia (11β,17,21-trihydroxy-4-pregnene-3,20-dione) or the closely related 21-hydroxy-20-keto pattern typical of corticosterone Ib (11β,21-dihydroxy-4-pregnene-3,20-dione). The numerous synthetic analogues of corticosteroids which appeared over the last twenty-five years deviate only little in their side chains. Indeed, all successful modifications left at least the C-20 carbonyl group intact (1). As part of our search for novel anti-inflammatory steroids it became of interest to try to find new biologically active replacements for the corticosteroid side chain. In the present paper, we report our surprising results in the 17α-alkynyl-11β,17-dihydroxyandrostane series.



I a) X = OH
b) X = H

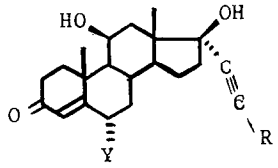
CHEMISTRY

The syntheses of the major compounds are summarized in schemes 1 to 3. Additional compounds are represented in scheme 4.



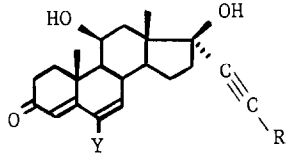
II a) Y = H
b) Y = CH_3

i) R - C ≡ C - M/THF
(M = Li, K or MgX)
ii) H_3O+
iii) DDQ/aq. acetone
iv) DDQ/C_6H_6



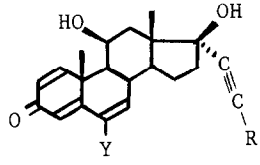
III

a) Y = H R = H (2) (3)
b) Y = H R = CH_3
c) Y = H R = C_2H_5
d) Y = H R = Ph
e) Y = H R = CH_2CH_2Ph
f) Y = H R = Cl (4)



IV

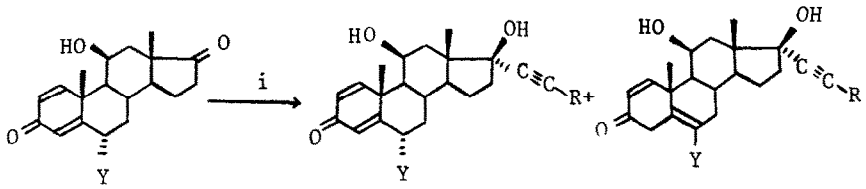
a) Y = H R = CH
b) Y = CH_3R = CH
c) Y = H R = Cl



V

a) Y = H R = CH_3
b) Y = CH R = CH_3
c) Y = H R = Cl
c) Y = CH_3R = Cl

Scheme 1



VI

a) Y = H
b) Y = CH_3

i = R-C≡C-M
(M = Li or K)

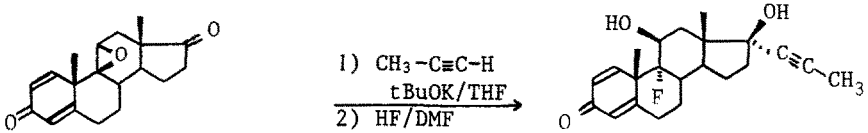
VII

a) Y = H R = CH_3
b) Y = H R = C_2H_5
c) Y = H R = Ph
d) Y = H R = $CH_3-C(=CH_2)-$
e) Y = H R = CF_3
f) Y = H R = Cl
g) Y = CH_3 R = CH_3

VIII

a) Y = H R = CH_3
b) Y = H R = Ph
c) Y = CH_3 R = CH_3

Scheme 2

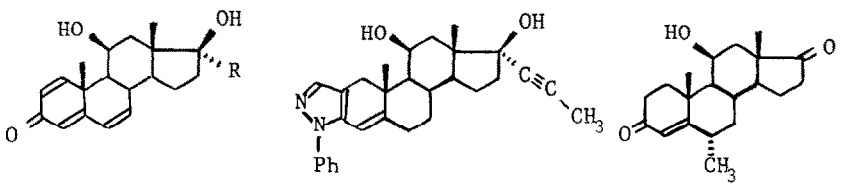


IX

1) $CH_3-C\equiv C-H$, tBuOK/THF
2) HF/DMF

X

Scheme 3



XI

a) R = $-CH_2-C\equiv C-H$
b) R = $-CH_2-CH=CH_2$

XII

XIII

Scheme 4

BIOLOGY

The experimental animals were Swiss mice, Sprague-Dawley rats, unless otherwise indicated, and New Zealand rabbits.

In the *in vitro* determinations, the compounds to be tested were dissolved in ethanol before diluting with water. For the oral route they were administered in an aqueous suspension containing 0.25% of carboxymethylcellulose and 0.20% of polysorbate 80. The ED_{50}s and EC_{50}s were calculated by a least square analysis.

Binding to cytoplasmic receptors

Organs of various species were used : uterus from mouse, 18 days old, uterus from estradiol-17β-primed rabbit, 50-55 days old, prostate from male rat, weighing 140-160 g and castrated for 24 hours, thymus and kidney from male rat, adrenalectomized for 4-7 days, for estrogen, progestin, androgen, glucocorticoid and mineralcorticoid receptors respectively. The first four organs were homogenized in 10 mM tris-HCl (pH 7.4) 0.25 M sucrose buffer with a tissue-buffer ratio (w/v) of 1:50 for uteri, 1:5 for prostate and 1:10 for thymus ; cytosols were obtained by centrifuging homogenates at 105,000 xg for 60 min at 4°C and incubated respectively with 5 nM of ^{3}H-estradiol-17β (58 Ci/mmole), 2.5 nM of ^{3}H-R5020 (17α,21-dimethyl-19-norpregna-4,9-diene-3,20-dione)(51.4 Ci/mmole),2.5 nM ^{3}H-R1881 (17-hydroxy-17α-methyl-4,9,11-estratrien-3-one) (58.2 Ci/mmole) for 2 hours at 0°C and with 5 nM ^{3}H-dexamethasone (9α-fluoro-16α-methyl-11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione) (25.9 Ci/mmole) for 4 hours at 0°C. Perfused kidneys were homogenized (1:3 w/v) in Krebs-Ringer phosphate buffer, pH 7.4, containing 1 g glucose per liter, and the homogenate was incubated with 2.5 nM ^{3}H-aldosterone for 30 min at 25°C and then centrifuged at 800 xg for 10 min at 0°C. All incubations were performed in the presence of 0 to 2500 nM unlabelled competing steroid. An 100 µl aliquot of incubated cytosol was stirred for 10 min at 0-4°C with 100 µl DCC (0.625% dextran T80 - 1.25% charcoal Norit-A) in a microtiter plate and then centrifuged for 10 min at 800 xg. The radioactivity of an 100 µl supernatant sample was measured. The percentage of bound radioligand was plotted against the logarithm of the radio-inert competitor concentration. The relative binding affinities (RBA) were calculated as described previously (5). The RBAs of estradiol, progesterone, testosterone, dexamethasone and aldosterone were taken to be equal to 100.

Anti-inflammatory activity in the cotton granuloma test

In this method, derived from that of Meier *et al.* (6), female Wistar rats, weighing about 100 g, in groups of 8 per dose, were implanted subscutaneously in the pectoral region with 2 pellets of cotton, each weighing 10 mg. The animals were sacrificed 2 days later and granulomas formed around the pellets were removed and weighed after drying for 18 hours at 60°C. The compounds were administered twice a day for 2 days, the first dosage being given just after the pellet implantation. The activity of the granuloma test was expressed as ED_{50}.

Thymolytic activity

Thymolytic activity was determined by weighing the thymus of the rats used in the granuloma test. The action was expressed as ED_{50}.

TABLE 1

In vitro and *in vivo* evaluation of
17α-alkynyl-11β,17-dihydroxyandrostane derivatives.

	R.B.A. (a)				ED_{50} mg/kg p.o. (b)			EC_{50} mg/ml (c)
	Gluco	Min	Prog	Andr	Granuloma	Thymus	Shock	Ear Edema
Ia cortisol	31	19	0	0	15	10	50	2.5
IIIa RU 200	5.1	0	2.9	1.7	IN 50 (d)	IN 50	IN 100	IN 1
b 25458	68	0.3	0.1	0.1	50	13	22	0.6
c 26275	94	0.9	1.7	0.2	50	20	50	0.7
d 26256	122	0.4	6.5	0.1	> 50	50	IN 50	> 1
e 26277	165	0.3	0.7	0	IN 50	50	IN 50	> 1
f 26136	61	0.1	2.2	0.7	40	30	IN 50	0.6
IVa RU 26946	58	0.1	0.4	0.1	40	10	16	> 1
b 27156	150	0.2	3	0.3	20	3.5	9	> 1
c 26751	70	0.2	1.4	0.2	40	30	50	> 1
Va RU 26988	127	0.5	0.3	0	10	4.5	9	0.07
b 28362	214	0.6	1.7	0.4	0.8	0.4	8	0.35
c 28086	166	0.7	1.3	0.1	9	3.5	15	1
d 28385	296	0.5	3.3	1.1	3.5	1	25	1
VIIa RU 26559	101	0.4	0.2	0	8	2.40	16	0.07
b 27066	126	0.6	0.5	0.1	20	1	IN 50	0.35
c 26936	143	0.2	3.5	0	IN 15	IN 15	IN 50	> 1
d 27083	162	1.1	0.4	0.1	12	5	IN 50	> 1
e 27070	137	1.6	0.4	0.2	4	0.12	9	0.30
f 28466	186	1.4	1	0.2	3	1	5	0.40
g 27143	167	0.2	1	0.3	2	0.05	50	> 1
VIIIa RU 27473	201	0.1	0.5	0.1	4	0.8	12	0.40
b 27013	246	0	1	0	50	10	IN 50	> 1
X RU 27155	135	2.3	1.8	0.1	15	0.50	20	0.40
XIa RU 27728	2.9	0.1	0	0	IN 50	IN 50	100	IN 1
b 27599	8.9	0	0	0	IN 50	IN 50	IN 100	IN 1
XII RU 27144	134	0	0	0	1.5	0.4	2.7	0.30
Prednisolone	47	18	0	0	3	1.50	60	0.50
Dexamethasone	100	17	0.4	0.5	0.05	0.035	1.7	0.07

(a) Relative binding affinities for the glucocorticoid (Gluco), the mineralocorticoid (Min), the progestin (Prog) and the androgen (Andr) receptors.

(b) ED_{50}: effective dose reducing granuloma or thymus weight by 50% as compared to controls. In the anaphylactic shock test, it is the dose reducing lethality by 50%.

(c) EC_{50}: effective concentration reducing the edema weight by 50% as compared to controls.

(d) IN : inactive at the dosage indicated.

Anti-allergic activity

Anti-allergic activity was evaluated by protection against anaphylactic shock. Groups of 10 male mice, weighing about 30 g, were sensitized with bovine serum albumin (BSA) by subplantar injection of 0.05 ml of an emulsion of BSA (10 mg/ml) in Freund adjuvant ; 8 days later the animals received 0.15 mg of BSA at the concentration of 1.5 mg/ml in physiological saline by i.v. route, and lethality was recorded 30 min afterwards. The compounds were administered 24 and 3 hours before the challenge. Again, activity was expressed in ED_{50}.

Dermal anti-inflammatory effect in the croton oil induced ear edema

This test, which is a modification of the rat model of Tonelli *et al.* (7), was carried out on groups of 8 female mice weighing 18-23 g. The edema was provoked on one ear by application of a solution of croton oil (2% v/v) in pyridine-water-ether 4:1:14.6 (by vol). Animals were sacrificed 6 hours later and the ears were removed and weighed. The edema was the difference of weight between the irritant-treated ear and the contralateral ear. The compound to be tested was dissolved in the croton oil solution and the EC_{50}, the effective concentration reducing the control edema values by 50%, was determined.

RESULTS AND DISCUSSION

The results are summarized in table 1. Reference compounds are represented by cortisol (Ia), prednisolone (11β,17,21-trihydroxy-1,4-pregnadiene-3,20-dione) and dexamethasone.

All the compounds represented in the table, with the exception of the known 17α-ethynyl derivative IIIa (2,3), displayed significant RBA's for the glucocorticoid receptor, with compound Vd having a maximum of three times the affinity of dexamethasone.

All derivatives possessed greatly reduced RBA's for the mineralocorticoid (aldosterone) receptor in contrast to cortisol, prednisolone or dexamethasone, constituting the first example of what could be considered as "pure glucocorticoids". ^{3}H-Va has already been used to determine glucocorticoid receptors in tissues where aldosterone receptors were present (8). Furthermore unlabeled Va (RU 26988) has been used to eliminate the interference of glucocorticoid binding in the determination of mineralocorticoid receptors (type I sites) by ^{3}H-aldosterone (9). Concerning the

in vivo activities, the most gratifying results were obtained in the topical antiinflammation assay (ear edema) with the 17α-propynyl derivatives IIIb, Va and VIIa, the last two compounds being equipotent with dexamethasone.

However, certain modifications which result in improved antiinflammatory activities in the corticosterone-cortisol series, failed in our hands. So the introduction ofa 9α-fluoro substituent (compare VIIa and X) or of a 6α-methyl substituent (VIIa versus VIIg) led to diminished topical antiinflammatory effectiveness while increasing the thymolytic effect. Several compounds also showed improved protection against anaphylactic shock as compared with prednisolone. This was the case especially for the pyrazolo derivative XII and the chloroethynyl derivative VIIf. That the position of the triple bond in the 17α-alkynyl substituent is essential was shown by the complete loss of affinity and *in vivo* activity of the 17α-propargyl derivative XIa. The allyl analogue XIb was also inactive.

CONCLUSION

Whereas structure-activity relationships do not appear straightforward in this series due to the multiplicity of activities generally associated with corticoid-like compounds, the relative binding affinities for the glucocorticoid receptor demonstrate the equivalency at the molecular level of the 17α-alkynyl-17β-hydroxy moiety and the classical corticoid side chain. This unexpected result as well as the fact that these compounds do not compete with aldosterone for the mineralocorticoid receptor indicate that they would be useful tools for the investigation of corticosteroid action. Moreover, the high topical antiinflammatory activity of RU 26559 (VIIa) and RU 26988 (Va) associated with reduced systemic effects suggest that these compounds would possibly be of therapeutic use.

EXPERIMENTAL PART

Elemental analyses were performed by the Analytical Department of Roussel-UCLAF. Melting points were taken on a Kofler hot stage and are uncorrected. Optical rotations were taken with a Roussel-Jouan Digital micropolarimeter. UV absorptions were measured in ethanol solution.

3-Ethoxy-11β-hydroxy-6-methyl-3,5-androstadien-17-one IIb

A suspension of 11β-hydroxy-6α-methyl-4-androstene-3,17-dione XIII (12.5 g) in absolute ethanol (65 ml) and ethyl orthoformate (13 ml), was heated to 60°C. While stirring, a solution of p-toluenesulfonic acid monohydrate (16 mg) in ethanol (1.6 ml) was added. Solution occurred within 1.5 min ; after a further 3 min triethylamine (2 ml) was added. The solution was cooled in an ice-bath and water (15 ml) was added dropwise. The formed crystals were separated by filtration, washed with cold ethanol-water 7:3 and dried at 60°C under reduced pressure. Yield : 11.0 g (81%) of IIb.

MP 205°C ; $[\alpha]_D$ - 76.5° ± 1.5° (1% in $CHCl_3$) ; λ max : 248-249 nm (19400)

Analysis : Calculated for $C_{22}H_{32}O_3$ C 76.70% H 9.36%
Found C 76.6% H 9.4%

11β,17β-Dihydroxy-21-methyl-17α-pregn-4-en-20-yn-3-one IIIb

3-Ethoxy-11β-hydroxy-3,5-androstadien-17-one (IIa, 3.4 g) in tetrahydrofuran (THF) (14 ml) was added to a stirred solution of 0.75 M propynylmagnesium bromide in THF (70 ml). After 45 min the reaction mixture was poured into cold 2 N aqueous HCl, stirred for 30 min and extracted with ether. Purification by chromatography through silica gel (elution with benzene-ethyl acetate 1:1) afforded 2.93 g (82%) of the desired compound IIIb.

MP 223°C (from isopropyl ether : methanol) ; $[\alpha]_D$ + 46° ± 2.5° (0.6% in EtOH) ; λ max : 241 nm (ε = 15300)

Analysis : Calculated for $C_{22}H_{30}O_3$ C 17.15% H 8.83%
Found C 76.8% H 8.8%

11β,17β-Dihydroxy-21-ethyl-17α-pregn-4-en-20-yn-3-one IIIc

Using the same starting materials as above (3 g) and a 0.8 M solution of butynyl magnesium bromide, 1.37 g (32%) of IIIc was obtained.

MP 170°C (from isopropyl ether) ; $[\alpha]_D$ + 49.5° ± 2.5° (0.5% chloroform) ; λ max : 241 nm (15400)

Analysis : Calculated for $C_{23}H_{32}O_3$ C 77.49% H 9.05%
Found C 77.3% H 9.2%

11β,17β-Dihydroxy-21-phenyl-17α-pregn-4-en-20-yn-3-one IIId

Phenylacetylene (7.2 ml) was added dropwise at 0°C to a stirred solution of 1.3 M n-butyllithium (40 ml). After addition was completed, 3-ethoxy-11β-hydroxy-3,5-androstadien-17-one (3 g) was added and the reaction mixture stirred for 17 hours at room temperature. After pouring into saturated aqueous ammonium chloride, and extraction with ether, the intermediate 3-ethoxy-21-phenyl-17α-pregna-3,5-diene-20-yn-11β,17β-diol was separated from unreacted starting material (700 mg) by silica gel filtration (eluent : benzene-ethyl acetate 8:2). Subsequent treatment with 25 ml 1 N aqueous HCl in 125 ml MeOH for 30 min at room temperature afforded after ether extraction and chromatography (eluent : benzene-ethyl

acetate 1:1) 1.1 g (30 %) of the pure noncrystalline compound IIId.
$[\alpha]_D$ - 4° ± 2° (0.7% in chloroform) ; λ max : 242 nm (33200)
Analysis : Calculated for $C_{27}H_{32}O_3$ C 80.16% H 7.97%
Found C 79.8% H 8.1%

11β,17β-Dihydroxy-21-(2-phenyl-ethyl)-17α-pregn-4-en-20-yn-3-one IIIe

Operating as above a 35% yield of the desired compound IIIe was obtained. (The Grignard reagent was prepared from 4-phenylbutyne and ethyl magnesium bromide).
MP 148°C (from isopropyl ether) ; $[\alpha]_D$ + 49.5° ± 2° (0.75% in chloroform) ; λ max : 241 nm (14700)
Analysis : Calculated for $C_{29}H_{36}O_3$ C 80.51% H 8.39%
Found C 80.68% H 8.4%

11β,17β-Dihydroxy-21-methyl-17α-pregna-4,6-dien-20-yn-3-one IVa

A solution of 3-ethoxy-11β-hydroxy-3,5-androstadien-17-one (3.45 g) in THF (14 ml) was added to a THF solution of propynyl magnesium bromide. The reaction mixture was stirred at RT for 45 min, poured into a cold aqueous solution of ammonium chloride, and extracted with ether. The organic phase was dried on sodium sulfate and the solvent was removed *in vacuo*. The crude residue was dissolved in acetone (95 ml), water (5 ml) and 4 g 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) was added at once, while stirring. After 1 hr at RT the reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted with methylene chloride. The organic phase was washed with 0.5 N sodium thiosulfate and again with a saturated sodium bicarbonate solution. The dried extract was purified by chromatography through silica gel (elution with benzene-ethyl acetate 1:1) affording 2.6 g (70%) of the desired dienone IVa.
MP 200°C (from isopropyl ether) ; $[\alpha]_D$ - 32.5° ± 1° (1% in $CHCl_3$)
Analysis : Calculated for $C_{22}H_{28}O_3$ C 77.60% H 8.29%
Found C 77.8% H 8.3%

11β,17β-Dihydroxy-6,21-dimethyl-17α-pregna-4,6-dien-20-yn-3-one IVb

Using the same procedure as for the preparation of IVa, starting with 3-ethoxy-11β-hydroxy-6-methyl-3,5-androstadien-17-one (6.3 g) 3.95 g (61%) of the desired amorphous dienone IVb was obtained.
$[\alpha]_D$ 0° in $CHCl_3$; λ max : 290 nm (22000)
Analysis : Calculated for $C_{23}H_{30}O_3$ C 77.93% H 8.53%
Found C 77.9% H 8.8%

21-Chloro-11β,17β-dihydroxy-17α-pregna-4,6-dien-20-yn-3-one IVc

The first step was performed as for the preparation of IIf starting with 3 g of 3-ethoxy-11β-hydroxy-3,5-androstadien-17-one.

Then, instead of hydrolysing with HCl, the reaction mixture was poured into aqueous ammonium chloride and extracted with ether. The dried extract was treated with DDQ as in the preparation of IVa affording, after the usual work up and purification, 2.05 g (63%) of the desired dienone IVc.
MP 236°C (from CH_2Cl_2-isopropyl ether)

11β,17β-Dihydroxy-21-methyl-17α-pregna-1,4,6-trien-20-yn-3-one Va

3-Ethoxy-11β-hydroxy-3,5-androstadien-17-one (3 g) was treated with a solution of propynyl magnesium bromide in THF as in the preparation of

IVa. The dried crude extract was dissolved in benzene (50 ml) and added to a stirred solution of DDQ (6.8 g) in benzene (200 ml). After 30 min, the reaction mixture was poured into aqueous sodium bicarbonate, and extracted repeatedly with ether. The organic phase was washed successively with an aqueous solution of 0.5 N sodium thiosulphate, and aqueous sodium bicarbonate. The solvent was removed under reduced pressure and the dried residue was purified by chromatography through silica gel (elution with benzene-ethyl acetate 1:1) affording 1.275 g (41.5%) of the desired trienone Va.

MP 218°C (from isopropyl ether) ; $[\alpha]_D$ - 108° ± 3° (0.4% in $CHCl_3$)
λ max : 221 nm (11200) 256 nm (9600) 299 nm (12700)

Analysis :			
Calculated for $C_{22}H_{26}O_3$	C 78.07%	H 7.74%	
Found	C 77.9%	H 7.7%	

11β,17β-Dihydroxy-6,21-dimethyl-17α-pregna-4,6-trien-20-yn-3-one Vb

Using the same procedure as for the preparation of Va, starting with 4.5 g of 3-ethoxy-11β-hydroxy-6-methyl-3,5-androstadien-17-one, 2.95 g (64%) of the desired trienone Vb was obtained.

MP 148°C (from MeOH) ; $[\alpha]_D$ - 86° ± 2° (1% in $CHCl_3$) ; λ max : 228 nm (12700) and 304 nm (10700)

Analysis :			
Calculated for $C_{23}H_{28}O_3$	C 78.37%	H 8.01%	
Found	C 78.6%	H 7.8%	

21-Chloro-11β,17β-dihydroxy-17α-pregna-1,4,6-trien-20-yn-3-one Vc

Using the same procedure as for the preparation of Va, starting with 3 g of 3-ethoxy-11β-hydroxy-3,5-androstadien-17-one, 1.555 g (35.5%) of the trienone Vc was obtained.

MP 250°C (from isopropyl ether-methanol) ; $[\alpha]_D$ - 132.5° ± 3° (0.45% in $CHCl_3$) ; λ max : 222 nm (11200) 257 nm (7700) and 299 nm (12700)

Analysis :			
Calculated for $C_{21}H_{23}O_3$	C 70.28%	H 6.46%	Cl 9.88%
Found	C 70.1%	H 6.3%	Cl 10.0%

21-Chloro-11β,17β-dihydroxy-6,21-dimethyl-17α-pregna- 1,4,6-trien-3-one Vd

Using the same procedure as for the preparation of Va, starting with 2.5 g of 3-ethoxy-11β-hydroxy-6-methyl-3,5-androstadien-17-one, 1.95 g (72%) of the trienone Vd was obtained. The compound was recrystallized in methanol giving a solvate of undefined MP (4% MeOH confirmed by NMR). $[\alpha]_D$ - 113° ± 3° (0.6% in $CHCl_3$) ; λ max : 227 nm (12400) 250 nm (8400) and 303 nm (10400)

Analysis :			
Calculated for $C_{22}H_{25}O_3Cl$, 0.5 MeOH			
	C 69.48%	H 6.99%	Cl 9.12%
Found	C 69.4%	H 6.8%	Cl 9.1%

11β,17β-Dihydroxy-21-methyl-17α-pregna-1,4-dien-20-yn-3-one VIIa

Propyne (dried over $CaCl_2$) was bubbled for 1 hour through an ice cooled solution of potassium t-butoxide (65.3 g) in THF (450 ml). To the white suspension which formed, hexamethylphosphorotriamide (HMPA, 20 ml) followed by 11β-hydroxy-1,4-androstadiene-3,17-dione (29.4 g) (10) was added. The reaction was stirred at RT for 20 hours, poured into 1.2 L aqueous ammonium chloride and extracted with ether. The dried extract was recrystallized from isopropyl ether-methanol 2:1 affording the deconjugated isomer VIIIa (22.48 g ; 68% yield).

This compound was treated with concentrated HCl (10 ml) in methanol (100 ml) at room temperature for 1 hour. The resulting solution was poured dropwise into 1 L ice-water while stirring. The colorless crystals were collected by filtration under reduced pressure, washed with water, and dried *in vacuo*, affording 21.6 g (65% overall yield) of the desired conjugated dienone VIIa (MP 236° - 240°C). Hydrolysis of the mother liquors from VIIIa followed by chromatography afforded an additional 6.5 g (total yield : 84.5%). Recrystallization from ethyl acetate yielded the analytical sample.

MP 240°C ; $[\alpha]_D$ - 5° ± 1° (1% in chloroform) ; λ max : 243 nm (ε 14300)

Analysis : Calculated for $C_{22}H_{28}O_3$ C 77.61% H 8.29%
Found C 77.5% H 8.3%

11β,17β-Dihydroxy-21-ethyl-17α-pregna-1,4-dien-20-yn-3-one VIIb

The procedure used for the preparation of VIIa was applied (butyne was used instead of propyne) yielding 67% of the desired compound VIIb.

MP 192°C ; $[\alpha]_D$ - 6.5° ± 1.5° (0.6% in $CHCl_3$) ; λ max : 243 - 244 nm (ε=14800).

Analysis : Calculated for $C_{23}H_{30}O_3$ C 77.93% H 8.53%
Found C 77.6% H 8.7%

11β,17β-Dihydroxy-21-phenyl-17α-pregna-1,4-dien-20-yn-3-one VIIc and 11β,17β-Dihydroxy-21-phenyl-17α-pregna-1,5-dien-20-yn-3-one VIIIb

Phenylacetylene (2.75 ml) was added to a suspension of potassium t-butoxide (2.91 g) in dioxane (100 ml) and stirred for 1 hour at RT. 11β-hydroxy-1,4-androstadiene-3,17-dione (3 g) was then added and the stirring was continued for 2 hours. After pouring into dilute HCl, and extraction with ether, the crude product was chromatographed through silica gel (eluent : benzene-ethyl acetate 6:4) affording 940 mg (23%) of the deconjugated dienone VIIIb

MP 210°C (forme ether)

Analysis : Calculated for $C_{27}H_{30}O_3$ C 80.56% H 7.51%
Found C 80.7% H 7.7%

and 1.265 g of the conjugated dienone VIIc (31%)

MP 262°C (from isopropyl ether) ; $[\alpha]_D$ - 21° ± 2° (0.7% in $CHCl_3$) ; λ max : 242 nm (33000)

Analysis : Found C 80.3% H 7.9%

along with recovered starting material (456 mg).

11β,17β-Dihydroxy-21-isopropenyl-17α-pregna-1,4-dien-20-yn-3-one VIId

Isopropenylacetylene (6 ml) was added dropwise to a solution of potassium t-butoxide (5.25 g) in THF (75 ml) at - 10°C.

11β-Hydroxy-1,4-androstadiene-3,17-dione (4.5 g) was added to the resulting yellowish suspension and the reaction mixture was stirred at 0°C for 2 hours, poured into dilute HCl and extracted with ether. The dried extract was chromatographed through silica gel (eluent benzene-ethyl acetate 1:1) affording 3.6 g (65.5%) of the desired compound VIId.

MP 218°C (from isopropyl-ether : methanol) ; $[\alpha]_D$ - 20° ± 1.5° (0.8% in $CHCl_3$) ; λ max : 235 nm (23100)

Analysis : Calculated for $C_{24}H_{30}O_3$ C 78.65% H 8.25%
Found C 78.4% H 8.4%

11β,17β-Dihydroxy-21-trifluoromethyl-17α-pregna-1,4-dien-20-yn-3-one VIIe

A solution of 11β-hydroxy-1,4-androstadiene-3,17-dione (2.1 g) in THF (35 ml) and HMPA (3.5 ml) was cooled to - 75°C and 1,1,1 trifluoro-2 propyne (30 mM) was bubbled into the solution. A solution of 3.4 g of potassium t-butoxide (28 mM) in THF (70 ml) and HMPA (3.5 ml) was then added dropwise, so as to maintain the temperature between - 75 and - 70°C (strongly exothermic reaction). The reaction medium darkened progressively becoming dark brown at the end of the addition. After pouring into aqueous ammonium chloride, extraction with ether afforded 4.4 g of crude material, which was filtered through silica gel (eluent : benzene-ethyl acetage 6:4) to yield 2.7 g (98%) of the desired compound VIIe.
MP 254 - 255°C (from isopropyl ether-methylene chloride) ;
$[\alpha]_D$ - 6.5° ± 1.5° (0.75% in $CHCl_3$) ; λ max : 242 nm (14900).

Analysis :	Calculated for $C_{22}H_{25}O_3F_3$	C 66.9%	H 6.39%	F 14.45%
	Found	C 67.1%	H 6.5%	F 14.1%

11β,17β-Dihydroxy-21-chloro-17α-pregna-1,4-dien-20-yn-3-one VIIf

Diisopropylamine (12.9 ml) was added dropwise to an ice cooled solution of 1.3 M n-butyllithium in hexane (70 ml) and THF (70 ml). The resulting solution of lithium diisopropylamide was cooled to - 75°C and cis dichloroethylene (4.5 ml) was added. The reaction mixture was allowed to return to RT and stirring was continued for 1 hour.

A red suspension of 11β-hydroxy-1,4-androstadiene-3,17-dione (5 g) and potassium t-butoxide (3.9 g) in THF (50 ml) and HMPA (5 ml) was then added under nitrogen. The reaction proceeded instantaneously. After 30 min, the reaction mixture was poured into chilled diluted HCl and extracted with ether. After evaporation of the solvent, the crude residue was dissolved in methanol (100 ml) and treated with aqueous 2 N HCl (20 ml) for 30 min at RT. Concentration under reduced pressure, addition of water and extraction with methylene chloride, followed by chromatography of the dried extract through silica gel (eluent : benzene-ethyl acetate 1:1) afforded 4.34 g (72%) of the desired compound VIIf.
MP 212°C (from methylene chloride-isopropyl ether) ; $[\alpha]_D$ - 12° ± 1° (0.9% in $CHCl_3$) ; λ max : 242 nm (14900).

Analysis :	Calculated for $C_{21}H_{25}O_3Cl$	C 69.89%	H 6.98%	Cl 9.83%
	Found	C 70.1%	H 7.3%	Cl 10.2%

11β,17β-Dihydroxy-6α,21-dimethyl-17α-pregna-1,4-dien-20-yn-3-one VIIg

Propyne (dried over $CaCl_2$) was bubbled for 1.5 hours through an ice cooled solution of potassium t-butoxide (10.3 g) in THF (200 ml).

To the white suspension which was formed, HMPA (20 ml), followed by 11β-hydroxy-6α-methyl-1,4-androstadiene-3,17-dione (5.75 g) (13) was added. The reaction mixture was stirred at RT for 8 hours, poured into water (1.5 L), acidified with 6 N aqueous HCl (40 ml) and extracted with ether. The dried extract was chromatographed through silica gel (elution with $CHCl_3$-acetone 8:2), affording successively :
- 600 mg (10%) of recovered starting material
- 742 mg (11.5%) of deconjugated dienone VIIIc
- 2.2 g (34%) of the desired conjugated dienone VIIg

MP 196°C (from isopropyl ether) ; $[\alpha]_D$ - 14° ± 2° (0.5% in $CHCl_3$) ;
λ max : 244 nm (14400).

Analysis : Calculated for $C_{23}H_{30}O_3$ C 77.93% H 8.53%
Found C 77.6% H 8.8%

11β,17β-Dihydroxy-21-methyl-17α-pregna-1,5-dien-20-yn-3-one VIIIa

Using the procedure for preparing VIIa but starting with 18 g of 11β-hydroxy-1,4-androstadiene-3,17-dione, 9.74 g of VIIIa were obtained after two recrystallizations from methanol. Chromatography of the mother liquors through silica gel (elution with benzene-ethyl acetate 1:1) afforded a further 3.25 g of the desired compound VIIIa. (total yield : 77.5%).

MP 226°C ; $[\alpha]_D$ - 27° ± 1.5° (1% in $CHCl_3$) ; λ max : 224 nm (11700).
Analysis : Calculated for $C_{22}H_{28}O_3$ C 77.61% H 8.29%
Found C 77.5% H 8.5%

11β,17β-Dihydroxy-9α-fluoro-21-methyl-17α-pregna-1,4-dien-20-yn-3-one X

Propyne (dried over $CaCl_2$) was bubbled for 1.5 hour through an ice-cooled solution of potassium t-butoxide (15.6 g) in THF (600 ml). To the white suspension which was formed, a solution of 9β,11β-oxido-1,4-androstadiene-3,17-dione (11) (4 g) in THF (40 ml) was added, and stirred at RT for 3 days. The reaction mixture was neutralized by addition of aqueous HCl, poured into water and extracted with ether. The dried extract (4.5 g) was dissolved in DMF (40 ml) and the solution was cooled to - 40°C. Anhydrous hydrofluoric acid in DMF (45 ml) (12) was added and the mixture allowed to return to room temperature. After 48 hours it was poured into chilled aqueous ammonia and the precipitate washed with water and dried, yielding 3.5 g of crude product. Ether extraction of the filtrate yielded a further 1.1 g. Chromatography through silica gel (eluent : CH_2Cl_2-isopropyl ether) afforded 1.12 g of the desired product X (23%).

MP 220°C and 260°C ; $[\alpha]_D$ - 2.5° ± 2° (0.5% in $CHCl_2$) ; λ max : 240 nm (14800).
Analysis : Calculated for $C_{22}H_{27}FO_3$ C 73.71% H 7.59% F 5.29%
Found C 73.5% H 7.7% F 5.0%

11β,17-Dihydroxy-17α-(prop-2-ynyl)-1,4,6-androstatrien-3-one XIa

Aluminum turnings (4 g) were suspended in THF (10 ml) and propargyl bromide (0.5 ml). The reaction was initiated with a trace of mercuric chloride and iodine. A solution of propargyl bromide (17 ml) in THF (100 ml) was then added dropwise so as to keep the temperature below 55°C. When the addition was complete, the reaction medium was cooled to 0°C and 3-ethoxy-11β-hydroxy-3,5-androstadien-17-one (6 g) was added at once. Stirring was continued for 30 min and triethylamine (15 ml) was added. The mixture was poured into a cold aqueous solution of sodium bicarbonate and extracted with ether. The dried extract was purified by chromatography through silica gel (elution with benzene-ethyl acetate 7:3) affording 3.6 g (54%) of the intermediate 3-ethoxy-17α-(prop-2-ynyl)-3,5-androstadiene-11β,17β-diol, which was used without further characterization as follows :

A solution of the compound (3.6 g) in benzene (50 ml) was added to a well-stirred solution of DDQ (4.8 g) in benzene (150 ml). Stirring was continued for 30 min and work up was performed as in the preparation of Va affording 1.8 g (55%, i.e. 30% overall) of the trienone XIa.

MP 210 - 212°C (from ethyl acetate) ; $[\alpha]_D$ + 1° ± 0.5° (1% in $CHCl_3$)
λ max : 221 nm (11600) 225 nm (9250) and 299 nm (12100).
Analysis : Calculated for $C_{22}H_{26}O_3$ C 78.07% H 7.74%
Found C 78.3% H 7.8%

17α-Allyl-11β,17-dihydroxy-1,4,6-androstatrien-3-one XIb

3-Ethoxy-11β-hydroxy-3,5-androstadien-17-one (3 g) was treated with a 0.8 molar solution of allyl magnesium bromide in ether. The usual work up using ammonium chloride afforded 17α-allyl-3-ethoxy-3,5-androstadiene-11β-17-diol, which was treated with DDQ without further characterization as in the preparation of Va, affording 1.73 g (56%) of the desired trienone XIb.
MP 212°C (from isopropyl ether) ; $[\alpha]_D$ 6.5° ± 2° (0.5% in $CHCl_3$)
λ max : 221 nm (11600) 255 nm (9250) 299 nm (12100)
Analysis : Calculated for $C_{22}H_{28}O_3$ C 77.6% H 8.28%
Found C 77.3% H 8.5%

17α-(Prop-1-ynyl)-2'-phenyl-2'H-androsta-2,4-dieno [3,2c] pyrazole-11β, 17-diol XII

11β,17β-Dihydroxy-21-methyl-17α-pregna-4-en-20-yn-3-one IIb (3.4 g) was added to a suspension of sodium hydride (1.5 g) in benzene (60 ml). Ethyl formate (15 ml) was then added dropwise while stirring. After the end of the addition, stirring was continued for 2 hours and the reaction mixture was poured into cold dilute aqueous HCl and extracted with methylene chloride. The dried extract (4.2 g) was dissolved in ethanol (100 ml) and a solution of phenylhydrazine hydrochloride (4.5 g) and sodium acetate (2.7 g) in 66 % aqueous ethanol (60 ml) was added. The solution was refluxed under nitrogen for 2.5 hours, cooled to RT, poured into water and extracted with methylene chloride. The solvent was removed under reduced pressure and the residue was dissolved in methanol (200 ml) and treated with 8 % aqueous sulfuric acid (35 ml) for 1 hour. Careful addition of saturated sodium bicarbonate solution (150 ml) led to precipitation of a brown material which was collected on a suction funnel, washed with water and dried. To saponify the formate esters which were partially formed in the first step, the crude material was treated with potassium hydroxide (5 g) in methanol (100 ml) at RT overnight. Extraction with methylene chloride after acidification followed by chromatography of the dried extract through silica gel (benzene-ethyl acetate 1:1) afforded 2.15 g (49%) of the desired compound XII
MP 188°C (from EtOH) ; $[\alpha]_D$ + 51° ± 2.5° (0.6% in $CHCl_3$) ; λ max : 261 nm (16900)
Analysis : Calculated for $C_{29}H_{34}N_2O_2$ C 78.69% H 7.74% N 6.33%
Found C 78.4% H 7.7% N 6.2%
along with the 1'-phenyl isomer (1.25 g ; 28%) which has not been fully characterized.

11β-Hydroxy-6α-methyl-4-androstene-3,17-dione XIII

11β,17β,21-Trihydroxy-6α-methyl-4-pregnene-3,20-dione (18 g) was suspended in 800 ml of 50% aqueous acetic acid. Sodium bismuthate (40g) was added and the reaction mixture was stirred at RT for 60 hours. After pouring into water (1.5 L) the mixture was extracted with chloroform. The organic phase was washed with saturated aqueous sodium bicarbonate, dried

on sodium sulfate and the solvent was removed under reduced pressure. On addition of methanol to the residue, the desired compound XIII crystallized out (9.97 g ; 65.5%). Chromatography of the mother liquors through silica gel Merck H (elution with $CHCl_3$-acetone 7:3) afforded another 2.05 g (total yield : 79%).

MP 231°C ; $[\alpha]_D$ + 175° ± 3.5° (0.5% in $CHCl_3$)

Analysis : Calculated for $C_{20}H_{28}O_3$ C 75.91% H 8.92%

Found C 75.8% H 9.0%

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