SYNTHESIS OF 2-CARBOXY-11β,17β-DIHYDROXY-17-METHYL-1,4-ANDROSTADIEN-3-ONE AND RELATED COMPOUNDS

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ABSTRACT

A series of 2-carboxy-1,4-androstadien-3-one derivatives and their alkyl esters, were prepared by high-yield syntheses. The compounds were structurally identified by physical methods.

All these steroids are characterized by a marked antiglucocorticoid activity that proved long-acting in the case of the ester derivatives.

2-Carboxy-11β,17β-dihydroxy-17-methyl-1,4-androstadien-3-one or roxibolone, and its n-decylester or decylroxibolone, are the most promising derivatives in consideration of their pharmacological properties.

INTRODUCTION

The biological activity of 11β,17β-dihydroxy-2-formyl-17-methyl-1,4-androstadien-3-one (3a) (formebolone) has been extensively investigated in the last ten years.

Formebolone is entirely free of any virilizing action (1,2,3,4), makes the nitrogen balance positive, counteracts the catabolic action of corticosteroid drugs (5,6,7,8,9), activates protein synthesis (10, 11,12) and energy-providing reactions (13,14). In vivo, the metabolism of formebolone results in two urinary metabolites derived respectively from a reduction and an oxidation of the aldehyde group (15): the replacement of the aldehyde function with a carboxyl or a

primary alcohol group could suppress the marked local irritating action. In this paper, we describe high-yield syntheses of 2-carboxy-1,4-androstadien-3-one derivatives, devoid of anabolic and virilizing effects but endowed with an antiglucocorticoid activity, markedly more tolerated and likely therefore to be used for the formulation of injectable long-acting compounds.

RESULTS

The most convenient route to 2-carboxy-11β,17β-dihydroxy-17-methyl-1,4-androstadien-3-one, roxibolone (4a), starts from the commercially available 11β-hydroxy-17α-methyltestosterone (1a). Its condensation with ethyl formate (15) gave 2-hydroxymethylene-11β-hydroxy-17α-methyl-testosterone (2a) in an almost quantitative yield. Dehydrogenation of this compound with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dioxane-methylene chloride gave 11β,17β-dihydroxy-2-formyl-17-methyl-1,4-androstadien-3-one (3a) (scheme 1). In an aqueous solution, both this product and its 11α-isomer are in equilibrium with the respective hydrated form at the formyl group (16). Similar behavior can be observed in alcoholic solutions. By UV spectroscopy, the two forms are characterized by two different chromophores with maxima at about 220 nm for the aldehydic form and 250 nm for the hydrated or hemiacetal forms.

The oxidation of 2-formyl-derivative 3a to the corresponding carboxylic acid 4a was performed with silver (I) oxide in a basic aqueous solution.

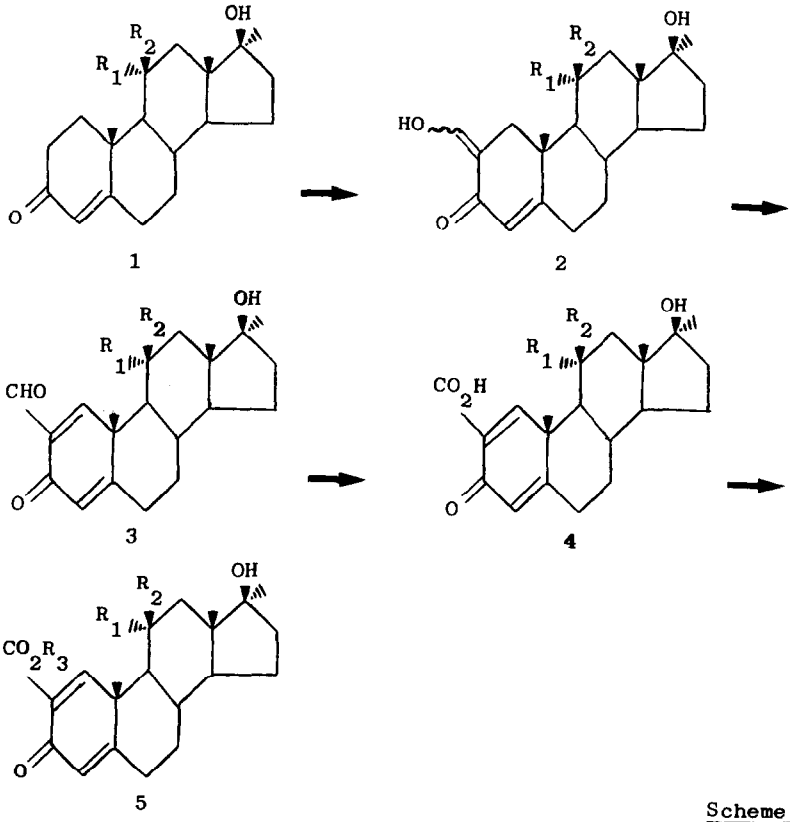
This synthetic sequence can be carried out with no purification of any intermediate and gives a 52% total yield. 2-Carboxy-11α,17β-dihydroxy-17-methyl-1,4-androstadien-3-one (4b) and 2-carboxy-17β-hydroxy-17-methyl-1,4-androstadien-3-one (4c) were obtained by a sim-

ilar oxidation of the corresponding 2-formyl derivatives 3b and 3c (table 1).

The 11-keto derivative 4d was obtained by the oxidation of 11α, 17β-dihydroxy-2-formyl-17-methyl-1,4-androstadien-3-one with chromic anhydride in pyridine according to Sarett (17).

Esters 5 were synthetized by alkylation of acids 4 with the corresponding alkyl iodides in the presence of silver (I) oxide (table 2).

However, the most convenient preparation of 2-carbodecoxy-11β, 17β-dihydroxy-17-methyl-1,4-androstadien-3-one (decylroxibolone) required a treatment of the roxibolone tetrabutylammonium salt with 1-iododecane in acetone at room temperature.



Scheme 1

TABLE 1

Compound No.	R_1	R_2	Empirical Formula	Yield %	mp (°C)	$[\alpha]_D^{+25}$	λ max (MaOH) ε / 10^4	IR (Nujol) cm^{-1}
4a*	H	OH	$C_{21}H_{28}O_5$	70	75 dec.	- 39.1° (c = 1 diox)	254 nm 1.045	3450, 1735, 1655, 1590
4b*	OH	H	$C_{21}H_{28}O_5$	71	66 dec.	-126.0° (c = 1 diox)	258 nm 1.190	3690, 3600, 1740, 1650, 1595
4c*	H	H	$C_{21}H_{28}O_4$	79	145 dec.	- 76.3° (c = 1 diox)	254 nm 1.100	3430, 3340, 1740, 1660, 1580
4d**	O	O	$C_{21}H_{26}O_5$	58	207 dec.	+127.9° (c = 1 pyrid)	248 nm 1.055	3520, 3358, 1740, 1705, 1668, 1578

* by oxidation with silver (I) oxide

** by oxidation with chromic anhydride

TABLE 2

Compound No.	R_1	R_2	R_3	Empirical Formula	Yield %	mp (°C)	Crystallization Solvent	IR cm^{-1}
5a	H	OH	CH_3	$C_{22}H_{30}O_5$	96	195 - 197	Ethyl Acetate	3580, 3370, 1740, 1660, 1620 (Nujol)
5b	OH	H	CH_3	$C_{22}H_{30}O_5$	95	188 - 190	Acetone/Petroleum Ether	3520, 3375, 1735, 1665, 1640 (Nujol)
5c	H	H	CH_3	$C_{22}H_{30}O_4$	90	144 - 146	Benzene	3450, 1735, 1660, 1620 (Nujol)
5d	O	O	CH_3	$C_{22}H_{28}O_5$	89	166 - 168	Ethyl Acetate/ Petroleum Ether	3500, 1740, 1710, 1660, 1630 (Nujol)
5e	H	OH	$n\text{-}C_4H_9$	$C_{25}H_{36}O_5$	75	159 - 161	Ethyl Acetate	3610, 3460, 1735, 1670, 1640 ($CHCl_3$)
5f	OH	H	$n\text{-}C_4H_9$	$C_{25}H_{36}O_5$	74	142 - 144	Ethyl Acetate	3485, 3410, 1740, 1655, 1630 (Nujol)
5g	H	OH	$n\text{-}C_8H_{17}$	$C_{29}H_{44}O_5$	85	74 - 78	Sublimation	3610, 3460, 1735, 1670, 1640 ($CHCl_3$)
5h	OH	H	$n\text{-}C_8H_{17}$	$C_{29}H_{44}O_5$	80	98 - 101	Ethyl Ether	3450, 3410, 1740, 1630, 1600 (Nujol)
5i	H	OH	$n\text{-}C_{10}H_{21}$	$C_{31}H_{48}O_5$	78	66 - 67	Sublimation	3610, 3460, 1735, 1670, 1640 ($CHCl_3$)
5l	OH	H	$n\text{-}C_{10}H_{21}$	$C_{31}H_{48}O_5$	80	82 - 84	Sublimation	3610, 3450, 1740, 1670, 1640 ($CHCl_3$)

DISCUSSION

Several 2-carboxy-17β-hydroxy-17-methyl-1,4-androstadien-3-one compounds (4) (scheme 1) were synthetized in order to study the effect of substituents at C-11. The 11β-hydroxy- derivative 4a, roxibolone or BR 906 (16), proved to be the most interesting compound being endowed with peculiar biologic properties.

This new compound, assessed by the classical test on the castrated rat (18) (Sprague-Dawley, male, bodyweight 100 g approximately), induced no significant changes in the weight of seminal vesicles, prostate and levator ani muscle (19); the lack of any virilizing action and of any activation of the skeletal muscles was definetely confirmed by the experimental evidence that roxibolone shows no affinity with the androgenic prostate and muscles receptors (20,21). The interest of this compound stems essentially from its antiglucocorticoid activity: in Sprague-Dawley rats it counteracts effectively the catabolic action (control of the nitrogen balance) (19) and the increased serum alkaline phosphatase levels (22) induced by a potent glucocorticoid agent such as dexamethasone-21-phosphate (11β,17 -dihydroxy-9-fluoro-16α-methyl-21-phosphonoxy-1,4-pregnadiene-3,20-dione).

Roxibolone, as well as its salts of alkaline or earth alkaline metals and quaternary ammonium salts, being soluble and stable in water, can be used for various pharmaceutical applications.

A series of alkyl esters (5) of carboxylic acids (4) was synthetized for the purpose of obtaining an optimal long-acting effect.

The resulting compounds differ in the nature of the substituent at C-11 and in the length of the alkylic chain of the alcohol bound to the carboxyl group. The biological activity of these esters (5) equals that of their original acids (4). In this group the decyl ester (5i), decylroxibolone or BR 917 (16), proved to be the most interesting derivative.

EXPERIMENTAL PART

Melting points were taken on a Buchi melting point apparatus, and are not corrected. Microanalyses were performed on a Perkin-Elmer 240 Elemental Analyzer. IR spectra were recorded with a Perkin-Elmer 257 spectrophotometer; ^{1}H-NMR spectra were recorded on a Varian XL-100 (100 MHz) instrument using tetramethylsilane as an internal standard and chemical shifts are reported as δ values. Optical rotations were determined with a Perkin-Elmer 141 polarimeter; U.V. spectra were recorded on a Perkin-Elmer 551 spectrophotometer. 11β-Hydroxy-17α-methyltestosterone was supplied by Schering. Pyridine was freshly distilled from barium oxide; benzene was distilled from sodium and methanol from magnesium. Ethyl formate RPE, Carlo Erba was used and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone was freshly crystallized from benzene/petroleum ether (mp 213-215°C).

2-Hydroxymethylene-11β-hydroxy-17α-methyltestosterone (2a)

Ethyl formate (184 ml) was added to a magnetically stirred solution of 11β-hydroxy-17α-methyltestosterone (1a) (100 g) in a mixture of pyridine (300 ml) and benzene (1000 ml). Then sodium hydride (70 g, 50% dispersion in mineral oil) was added portionwise and methanol (50 ml) was slowly added dropwise, with the temperature kept below 30°C. The reaction mixture was stirred under a nitrogen atmosphere for 4 hours. After this time methanol (100 ml) and water (800 ml) were added with caution. The aqueous layer was separated and the organic phase was extracted with a solution of 2% sodium hydroxide (3 x 250 ml). The combined aqueous layers were washed with methylene chloride (3 x 270 ml), acidified to pH 2 with 12% hydrochloric acid and extracted with methylene chloride (4 x 300 ml). The combined organic layers, dried with sodium sulfate and evaporated under reduced pressure, gave 110 g of crude 2a which was directly used in the subsequent reaction.

mp (AcOEt/C_6H_6) 138-140°C

$C_{21}H_{30}O_4$ (MW 346.45)

theoretical : C 72.80% H 8.73%

experimental: C 72.92% H 8.70%

IR (Nujol) 3340, 2705, 1640, 1567, 1455 cm^{-1}

^{1}H-NMR ($CDCl_3$) 1.17 (s, 3H, CH_3-$C_{(13)}$); 1.20 (s, 3H, CH_3-$C_{(10)}$); 1.37 (s, 3H, CH_3-COH); 4.43 (m, 1H, CHOH); 5.74 (bs, 1H, $C_{(14)}H$);

7.38 (bs, 1H, C=CHOH)

UV (MeOH) 256 nm, $\varepsilon = 9.800 \cdot 10^3$; 312 nm, $\varepsilon = 6.010 \cdot 10^3$

$[\alpha]_D^{20}$ + 5.71 (c = 1, chloroform)

11β,17β-Dihydroxy-2-formyl-17-methyl-1,4-androstadien-3-one (3a)

A solution of 2-hydroxymethylene-11β-hydroxy-17α-methyltestosterone (2a) (110 g) in dioxane (600 ml) was added to a solution of DDQ (70 g) in dioxane (800 ml). The mixture was stirred for 90 min, subsequently diluted with methylene chloride (1600 ml) and stirring was continued for 60 min. The solid product was filtered off; the organic layer was washed first with a saturated sodium bicarbonate solution (4 x 500 ml), subsequently with brine (2 x 500 ml), and finally dried with sodium sulfate. Evaporation under reduced pressure yielded 100 g of crude 3a which was used as such in the subsequent reaction.

mp (Et_2O/ petroleum ether) 101-103°C

$C_{21}H_{28}O_4$ (MW 344.43)

theoretical : C 73.23% H 8.19%

experimental: C 73.20% H 8.15%

IR (Nujol) 3420, 1710, 1695, 1643, 1605 cm^{-1}

^{1}H-NMR ($CHCl_3$) 1.19 (s, 3H, CH_3-$C_{(13)}$); 1.23 (s, 3H, CH_3-$C_{(10)}$); 1.63 (s, 3H, CH_3-COH); 4.45 (m, 1H, CHOH); 6.08 (s, 1H, $CH_{(14)}H$); 8.10 (s, 1H, $C_{(1)}H$); 10.27 (1H, s, CHO)

UV (MeOH) 226 nm, $\varepsilon = 2.290 \cdot 10^4$

(H_2O) 220 nm, $\varepsilon = 1.912 \cdot 10^4$ (in both cases an inflection at about 255 nm was observed)

$[\alpha]_D^{20}$ + 30.9 (c = 1, chloroform)

2-Carboxy-11β,17β-dihydroxy-17-methyl-1,4-androstadien-3-one

A solution of silver nitrate (70g) in water (600ml) was added drop-

wise to a magnetically stirred solution of sodium hydroxide (46 g) in water (600 ml) under nitrogen atmosphere. 11β,17β-Dihydroxy-2-formyl-17-methyl-1,4-androstadien-3-one (3a) (100 g) was subsequently added portionwise. After 4 hours at room temperature, the solid product was filtered off and washed with water. The aqueous layer was washed with methylene chloride (3 x 250 ml) and acidified to pH 3 with 2N hydrochloric acid. After a new extraction with methylene chloride (4 x 350 ml), the combined organic layers were extracted in turn with a saturated sodium bicarbonate solution (3 x 400 ml). The combined aqueous phases were acidified to pH 3 with 2N hydrochloric acid and extracted with methylene chloride (4 x 300 ml). The combined organic layers were dried with sodium sulfate and evaporated under reduced pressure. The residue was decolorized by filtration through kieselgel. The resulting 2-carboxy-11β,17β-dihydroxy-17-methyl-1,4-androstadien-3-one (4a) was dissolved in saturated sodium bicarbonate solution (1000 ml) and precipitated by acidification to pH 2; 58.0 g of pure product was obtained (52.1% yield).

$C_{21}H_{28}O_5$ (MW 360.43)

theoretical : C 69.97% H 7.83%

experimental: C 69.89% H 7.68%

^{1}H-NMR (C_5D_5N) 1.33 (s, 3H, $C\underline{H}_3$-$C_{(13)}$); 1.51 (s, 3H, $C\underline{H}_3$-$C_{(10)}$); 1.65 (s, 3H, $C\underline{H}_3$-COH); 4.45 (s, 1H, C$\underline{H}$OH); 6.20 (s, 1H, $C_{(4)}\underline{H}$); 8.75 (s, 1H, $C_{(1)}\underline{H}$).

2-Carboxy-17β-hydroxy-17-methyl-1,4-androstadiene-3,11-dione

A solution of 11α,17β-dihydroxy-2-formyl-17-methyl-1,4-androstadien-3-one (23) (10.3 g) in pyridine (250 ml) was added to a solution of chromic anhydride (7 g) in pyridine (70 ml). After 24 hours at room temperature the precipitate was filtered off and washed with ether (1000 ml). Chromatography on kieselgel of the residue, obtained after evaporation under reduced pressure, afforded 6.3 g of compound 4d (58.7% yield).

$C_{21}H_{26}O_5$ (MW 358.42)

theoretical : C 70.37% H 7.31%

experimental: C 70.48% H 7.39%

^{1}H-NMR (CD_3SOCD_3) 0.76 (s, 3H, $C\underline{H}_3$-$C_{(13)}$); 1.16 (s, 3H, $C\underline{H}_3$-$C_{(10)}$); 1.47 (s, 3H, $C\underline{H}_3$-COH); 6.22 (s, 1H, $C_{(4)}\underline{H}$); 8.44 (s, 1H, $C_{(1)}\underline{H}$).

2-Carbomethoxy-11β,17β-dihydroxy-17-methyl-1,4-androstadien-3-one (5a)

Methyl iodide (13.0 ml) and silver (I) oxide (12.5 g) were added, in order, to a solution of 2-carboxy-11β,17β-dihydroxy-17-methyl-1,4-androstadien-3-one (4a) (10.4 g) in methylene chloride (300 ml). The mixture was magnetically stirred for 1 hour at room temperature, and then filtered through a celite cake. The filter cake was washed with methylene chloride (3 x 100 ml) and the combined organic layers evaporated under reduced pressure. Recrystallization from ethyl acetate gave 10.0 g of compound 5a (96% yield).

$C_{22}H_{30}O_5$ (MW 374.46)

theoretical : C 70.55% H 8.08%

experimental: C 70.65% H 8.00%

^{1}H-NMR ($CDCl_3$) 1.17 (s, 3H, CH_3-$C_{(13)}$); 1.20 (s, 3H, CH_3-$C_{(10)}$); 1.57 (s, 3H, CH_3-COH); 3.87 (s, 3H, CH_3-OCO); 3.47 (m, 1H, CHOH); 6.06 (bs, 1H, $C_{(4)}$-H); 7.97 (s, 1H, $C_{(1)}$-H)

UV (MeOH) 245 nm, $\epsilon = 1.235 \cdot 10^4$

$[\alpha]_D^{20}$ + 15.8 (c = 1, chloroform)

2-Carbodecoxy-11β,17β-dihydroxy-17-methyl-1,4-androstadien-3-one (5i)

A solution of 2-carboxy-11β,17β-dihydroxy-17-methyl-1,4-androstadien-3-one (4a) (60 g) in tetrabutylammonium hydroxide (900 ml, 2.77% aqueous solution) was lyophilized. 1-Iododecane (40 ml) was added to a solution of the lyophilized product in acetone (350 ml). The reaction mixture was allowed to stand at room temperature for 72 hours, diluted with ether (900 ml) and filtered. Chromatography on kieselgel of the residue, obtained from the evaporation under reduced pressure of the organic phase, afforded 68.0 g of compound 5i (78% yield).

$C_{31}H_{48}O_5$ (MW 500.68)

theoretical : C 74.36% H 9.66%

experimental: C 74.54% H 9.82%

^{1}H-NMR ($CDCl_3$) 0.93 (d, 3H, CH_3-CH_2); 1.16 (s, 3H, CH_3-$C_{(13)}$); 1.18 (s, 3H, CH_3-$C_{(10)}$); 1.53 (s, 3H, CH_3-$C_{(17)}$); 4.10-4.61 (m, 3H, CHOH, CH_2-OCO); 6.03 (s, 1H, $C_{(4)}$-H); 7.90 (s, 1H, $C_{(1)}$-H)

UV (MeOH) 246 nm, $\epsilon = 1.056 \cdot 10^4$

$[\alpha]_D^{20}$ + 5.3 (c = 1, chloroform).

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