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GAS–LIQUID CHROMATOGRAPHIC STUDIES OF REACTIONS AND STRUCTURAL RELATIONSHIPS OF STEROIDS

PART III. 11α-HYDROXYSTEROIDS OF THE ANDROSTANE AND PREGNANE SERIES*

FRANTZ A. VANDENHEUVEL

Animal Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ontario K1A OC6 (Canada)

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SUMMARY

Qualitative and quantitative effects of classical reactions on steroids observed by gas–liquid chromatography (GLC) under standardized conditions, including the double internal-standard technique, are reported. Simple procedures applicable to nanogram amounts of reactants which afford excellent yields of the major products are described. Reactions studied include the Wolff–Kishner removal of keto groups, their conversion into hydroxyl groups with sodium–ethanol or sodium borohydride and into dioxolone derivatives with ethylene glycol; the conversion of hydroxyl into keto groups with chromium trioxide and to trimethylsilyl (TMS) ethers by hexamethyldisilazane; the hydrolysis of dioxolone and TMS derivatives by H^+. Gas–liquid chromatograms of reaction mixtures of single- and multistep reactions readily provide information on the effects on the 11α-hydroxy and other functional groups at positions 3 and 17 (androstane series) and positions 3 and 20 (pregnane series), and the retention times of many steroids unavailable from commercial or other sources. GLC data analysis provides relationships between steroid structure and retention time from which methods for the computation of retention times and for steroid identification are designed. The accuracy of the calculation methods is demonstrated.

INTRODUCTION

A systematic survey of steroidal hormones, precursors and metabolites in domestic animals undertaken in this laboratory required access to relevant steroid standards for the purpose of identification. As this study included poorly explored domains of steroid metabolism, the choice of standards was given the widest possible scope. Chemical and chromatographic properties of standards obtained by synthesis or from commercial and other sources were systematically observed under highly

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standardized, reproducible conditions, and their value in steroid characterization was thoroughly assessed. Such properties have already been reported in Parts I and II of the present series for steroids of the androstane series substituted at the 3, 11 and 17 positions[1], and for steroids of the pregnane series substituted at the 3, 11 and 20 positions[2]. The 232 relevant steroids, which did not include the 11α-hydroxy species, comprised numerous hormones and known metabolites. In contrast, the 77 corresponding 11α-hydroxysteroids reported in the present paper include relatively few recognized metabolic intermediates, for example, 11α-hydroxyprogesterone. Although such compounds were readily obtainable by alkali metal reduction of 11-oxosteroids[3,4], reported preparations were few, and available standards even less numerous. In syntheses described below, the required standards were obtained by sodium–ethanol reduction (RN) of 11-oxosteroids, along with much information on their chemical properties and the possible use of the reaction for the characterization of 11-substituted steroids. Gas–liquid chromatography (GLC) of the products showed that G_R-oddity, as defined in ref. 1, was unusually extensive in these compounds, and that, in contrast to other steroid species, it was positive in most cases. This paper will show that, in spite of extraordinary GLC properties, 11α-hydroxysteroids form closely related groups[1]. Hence, strong additional evidence will be provided for the general applicability of steroid structure–retention time relationships[1] whereby accurate retention times can be predicted, and for the reliability of the calculated values in steroid identification[1,2].

Definitions of abbreviations and symbols used below will be found in Part I of the present series of articles[1].

EXPERIMENTAL

Reactions

Most of the syntheses described in Diagrams 1—9 were carried out by first applying the RN procedure described below to dioxolone (DO) derivatives featuring a free (11) group. The syntheses of these DO derivatives have been described[1,2]. Procedures used in subsequent hydrolysis of DO derivatives (HY), reduction of keto groups by sodium borohydride (RD), Wolff–Kishner removal of keto groups (WK), and chromium trioxide oxidation of hydroxyl groups (OX) have been described in detail[1].

RN. From 0 to 1 mg of steroid placed in a 15-ml §10 centrifuge tube was dissolved in 250 μl of absolute ethanol. After filling the tube with nitrogen, a small sliver of clean sodium was added, as described below, and the reaction allowed to proceed for a few minutes until hydrogen evolution had ceased. Unreacted sodium, if any, was dissolved by adding 250 μl of ethanol. The contents were carefully neutralized with 1 *N* acetic acid measured from a burette (blue spot on wet Congo red paper), and extracted three times with 1 ml of chloroform. The total extract, washed with 500 ml of water, was evaporated to dryness under a stream of nitrogen.

General extraction and washing procedures described in ref. 1 were used. Clean sodium slivers were obtained from a small block of sodium wedged at the bottom of a 100-ml weighing bottle, washed several times with pure hexane, and kept in hexane, under nitrogen, in the stoppered bottle until used. After scratching a small area of the block surface clear of oxide with a scalpel, a sliver was removed

by scraping with the tip of a 0.5-mm-diameter stainless-steel wire to which it stuck and immediately transferred to the reaction tube. The size of this sliver, estimated from previous tests, corresponded to at least 5 ml of acid.

GLC and thin-layer chromatography (TLC)

Both methods were used as previously described[1,2]. TLC was used extensively as a purification step particularly in the synthesis of pregnane derivatives for reasons already discussed[2]. Trimethylsilyl (TMS) derivatization of all 11α-hydroxysteroids was carried out in microtubes filled with nitrogen and heated to 30–35° to ensure complete reaction (*cf.* Discussion).

THE DATA

Table I and Diagrams 1–9 describe syntheses of 11α-hydrosteroids from (11)-featuring compounds, the sources of which are indicated. Percentages of main products only are given for successive reactions indicated to the left or middle of the diagrams by the appropriate symbols. Retention times t'_{NR} of TMS derivatives are shown preceded by D followed by the time in $10^{-2} \times$ min and preceded by D,N when this time was the same whether the reaction mixture was derivatized or not. The retention time is followed by the corresponding L_R value in parentheses.

$$L_R = 10^3 \cdot \log t'_{NR} \qquad \text{(eqn. 6 in ref. 1)}$$

TABEL I

EFFECT OF NASCENT HYDROGEN GENERATED BY SODIUM DISSOLVING IN ETHANOL (RN) ON UNSATURATED STEROIDS OF THE ANDROSTANE SERIES

Starting material		*Main product(s)**	
Abbreviation	*Source and GLC properties*	*Abbreviation*	*GLC properties*
Δ14A3β17β	Ref. 1, Table X	*cf.* text	
Δ15A3β17β	Ref. 1, Table X	Δ15A3β17β	Ref. 1, Table X
5βA17β(3)	Ref. 1, Table X	5βA3α17β	Ref. 1, Table X
5αA17β(3)	Ref. 1, Table X	5αA3β17β	Ref. 1, Table X
Δ14A17β(3)	Ref. 1, Table X	5αA3β17β; *cf.* text	
5αA3α(17)	Ref. 1, Table IX	5αA3α17β	Ref. 1, Table X
5αA(3,17)	Ref. 1, Table IX	5αA3β17β	Ref. 1, Table X
Δ14A(3,17)	Ref. 1, Table IX	5αA3β17β; *cf.* text	
5βA(11)	Ref. 1, Table III	5βA11α	This article, Table IV
5αA(11)	Ref. 1, Table III	5αA11α	This article, Table IV
5αA3α(11)	Ref. 1, Table III	5αA3α11α	This article, Table IV
5βA3α(11)	Ref. 1, Table III	5βA3α11α	This article, Table IV
5αA3β(11)	Ref. 1, Table III	5αA3β11α	This article, Table IV
Δ5A3β(11)	WK reduction of Δ5A3β(11,17)	Δ5A3β11α	This article, Table IV
5βA(11,17)	Ref. 1, Table V	5βA11α17β	This article, Table VI
5αA(11,17)	Ref. 1, Table V	5αA11α17β	This article, Table VI
5βA3α(11,17)	Ref. 1, Table V	5βA3α11α17β	This article, Table VI
5αA3β(11,17)	Ref. 1, Table V	5αA3β11α17β	This article, Table VI
Δ5A3β(11,17)	SRC	Δ5A3β11α17β	This acticle, Table VI

* Except in cases involving a Δ4A-compound, yields were in the 85–95% range with a large excess of sodium (*cf.* text).

A	B
5β-A(11)DO(17)*	5α-A(11)DO(17)**
N,D 315 (2500)	N,D 349 (2543)
RN	
90	**89**
5β-A11α-DO(17)	5α-A11α-DO(17)
D 350 (2544)	D 363 (2560)
ΔDO(17) = 173***	ΔDO(17) = 178***
HY	
90	**88**
5β-A11α(17)	5α-A11α(17)
D 235 (2371)	D 241 (2382)
RD	
90	**85**
5β-A11α17β	5α-A11α17β
D 265 (2423)	D 273 (2436)

Diagram 1. Synthesis of 5β-A11α(17), 5α-A11α(17), 5β-A11α17β and 5α-A11α17β.
* For the preparation of this compound, *cf.* ref. 1, Diagram 16.
** For the preparation of this compound, *cf.* ref. 1, Diagram 12.
*** ΔDO(17) is the difference between the L_R values of the TMS derivatives of the (17)-steroid and its dioxolone derivative.

A	B
5β-A3α(11)DO(17)*	5α-A3α(11)DO(17)**
D 602 (2779)	D 621 (2793)
RN	
85	**92**
5β-A3α11α-DO(17)	5α-A3α11α-DO(17)
D 632 (2801)	D 578 (2762)
ΔDO(17) = 175***	ΔDO(17) = 176***
HY	
87	**90**
5β-A3α11α(17)	5α-A3α11α(17)
D 422 (2626)	D 386 (2586)

WK	RD	WK	RD
85	**88**	**95**	**91**
5β-A3α11α	5β-A3α11α17β	5α-A3α11α	5α-A3α11α17β
D 251 (2399)	D 467 (2669)	D 229 (2360)	D 441 (2644)
R_b = 0.326	R_b = 0.026	R_b = 0.323	R_b = 0.033

Diagram 2. Synthesis of 5β-A3α11α(17), 5α-A3α11α(17), 5β-A3α11α17β and 5α-A3α11α17.
* For the preparation of this compound, *cf.* ref. 1, Diagram 16.
** For the preparation of this compound, *cf.* ref. 1, Diagram 12.
*** ΔDO(17) is the difference between L_R values of the TMS derivatives of the (17)-compound and its dioxolone derivative.

A		B
5αA3β(11,17)*		Δ5A3β(11,17)**
D 440 (2643)		D 427 (2627)
	DO	
95		**95**
5αA3β(11)DO(17)		Δ5A3β(11)DO(17)
D 801 (2904)		D 768 (2885)
ΔDO(17) = 261***		ΔDO(17) = 258***
	RN	
90		**92**
5αA3β11αDO(17)		Δ5A3β11αDO(17)
D 766 (2884)		D 745 (2872)
ΔDO(17) = 180***		ΔDO(17) = 176***
	HY	
86		**89**
5αA3β11α(17)		Δ5A3β11α(17)
D 506 (2704)		D 496 (2696)

WK	RD	WK	RD
88	**83**	**88**	**90**
5αA3β11α	5αA3β11α17β	Δ5A3β11α	Δ5A3β11α17β
D 300 (2477)	D 574 (2759)	D 295 (2470)	D 567 (2753)

Diagram 3. Synthesis of 5αA3β11α(17), Δ5A3β11α(17), 5αA3β11α17β and Δ5A3β11α17β.

* For preparation of this compound, *cf.* ref. 1, Table V.

** Obtained from SRC.

*** ΔDO(17) is the difference between the L_R values of TMS derivatives of the (17)-compound and its dioxolone derivative.

	A	B	C
	5βA(3,11,17)*	5αA(3,11,17)*	Δ4A(3,11,17)*
	D,N 348 (2541)	D,N 379 (2578)	D,N 426 (2629)
DO			
	95	**93**	**95****
	5βA(11)DO(3,17)	5αA(11)DO(3,17)	Δ4A(11)DO(3,17)?
	D,N 947 (2978)	D,N 1064 (3027)	D,N 1030 (3013)
	ΔDO(3,17) = 435***	ΔDO(3,17) = 449***	ΔDO(3,17) = 384***
RN, HY§			
	80	**85**	**87**
	5βA11α(3,17)	5αA11α(3,17)	Δ4A11α(3,17)
	D 494 (2693)	D 498 (2697)	D 616 (2790)
RD			
	80	**85**	**85**
	5βA3α11α17β	5αA3β11α17β	Δ4A3β11α17β
	D 467 (2669)	D 574 (2759)	D 548 (2739)

Diagram 4. Synthesis of 5βA11α(3,17), 5αA11α(3,17), Δ4A11α(3,17), 5βA3α11α17β, 5αA3β11α17β and Δ4A3β11α17β.

* For sources of this compound, *cf.* ref. 1, Table V.

** Yield and nature of this product are discussed in text.

*** ΔDO(3,17) is the difference between the L_R values of TMS derivatives of the (3,17)-compound and its dioxolone derivative.

§ Neutralization of RN reduction mixture was carried out with dilute HCl: *cf.* text.

A	B
5βP(11)*	5αP(11)**
D,N 184.5 (2266)	D,N 201 (2303)
RN	
90	**92**
5βP11α	5αP11α
D 211.5 (2325)	D 215 (2332)

Diagram 5. Synthesis of 5βP11α and 5αP11α.
* For preparation of this compound, *cf.* ref. 2, Diagram 4.
** For preparation of this compound, *cf.* ref. 2, Diagram 5.

	A	B	C
	5βP3β(11)DO(20)*	5βP3α(11)DO(20)*	5αP3β(11)DO(20)*
	D 1152 (3061)	D 1132 (3053)	D 1446 (3160)
	R_b = 0.700	R_b = 0.700	R_b = 0.700
RN**			
	90	**91**	**92**
	5βP3β11αDO(20)	5βP3α11αDO(20)	5αP3β11αDO(20)
	D 1066 (3027)	D 1073 (3030)	D 1301 (3115)
	ΔDO(20) = 253***	ΔDO(20) = 240***	ΔDO(20) = 245***
	R_b = 0.299	R_b = 0.249	R_b = 0.279
HY**			
	92	**95**	**90**
	5βP3β11α(20)	5βP3α11α(20)	5αP3β11α(20)
	D 608.5 (2784)	D 617 (2790)	D 741 (2870)
	R_b = 0.246	R_b = 0.191	R_b = 0.201
	WK — RD (2 h)	WK — RD (2 h)	WK — RD (2 h)
	90 **62**	**90** **64**	**92** **60**
	5βP3β11α 5βP3β11α20β	5βP3α11α 5βP3α11α20β	5αP3β11α 5αP3β11α20β
	D 374 (2572) D 930 (2968)	D 380 (2579) D 935 (2970)	D 452 (2655) D 1120 (3049)
	+	+	+
	30	**30**	**30**
	5βP3β11α20α	5βP3α11α20α	5αP3β11α20α
	D 844 (2926)	D 852 (2930)	D 1022 (3009)

Diagram 6. Synthesis of 5βP3β11α, 5βP3β11α(20), 5βP3β11α20β, 5βP3β11α20α and homologuous 5βP3α- and 5αP3β-steroids.
* For preparation of this compound, *cf.* ref. 2, Diagram 7.
** All reactions were carried out with purified material extracted from TLC zones of R_b values indicated.
*** ΔDO(20) is the difference between L_R values of TMS derivatives of 11α(20)-steroid and its dioxolone derivative.

A	B	C
5β-P(11)DO(3,20)*	5α-P(11)DO(3,20)*	Δ14-P(11)DO(3,20)?§§
D 1740 (3240)	D 1926 (3284)	D 1858 (3269)
R_b = 0.950	R_b = 0.950	R_b = 0.950
RN**		
95	**95**	**97**
5β-P11α-DO(3,20)	5α-P11α-DO(3,20)	Δ14-P11α-DO(3,20)?§§
D 1772 (3248)	D 1816 (3259)	D 1816 (3259)
ΔDO(3,20) = 390***	ΔDO(3,20) = 395***	ΔDO(3,20) = 301***
R_b = 0.738	R_b = 0.805	R_b = 0.788
HY**		
94	**97**	**96**
5β-P11α(3,20)	5α-P11α(3,20)	Δ14-P11α(3,20)
D 721 (2858)	D 732 (2864)	D 908 (2958)
R_b = 0.577	R_b = 0.591	R_b = 0.446
WK **RD** (2 h)	**WK** **RD** (2 h)	**WK**§ **RD** (2 h)
90 **64**	**93** **62**	**60**
5β-P11α 5β-P3α11α20β	5α-P11α 5α-P3β11α20β	Δ14-P3β11α20β
D 211.5 (2325) D 935 (2970)	D 215 (2332) D 1120 (3049)	D 1075 (3033)
+	+	+
35	**31**	**31**
5β-P3α11α20α	5α-P3β11α20α	Δ14-P3β11α20α
D 844 (2926)	D 1022 (3009)	D 976 (2989)

Diagram, 7. Synthesis of 5β-P11α(3,20), 5α-P11α(3,20), Δ14-P11α(3,20), Δ14-P3β11α20β and Δ14-P3β11α20α. Products obtained by WK and RD reactions confirm identity of (3,20)-steroids.

* For preparation of this compound, *cf.* ref. 2, Diagram 8.

** All reactions were carried out with purified material extracted from TLC zones of R_b value indicated. Product concentrations refer to material extracted from TLC zones of R_b value indicated.

*** ΔDO(3,20) is the difference of L_R values of TMS derivatives of the 11α(3,20) steroid and its dioxolone derivative.

§ Abnormal reaction: *cf.* text.

§§ The identity of this compound is discussed in text.

A	B
5β-P(11)DO(3)*	5α-P(11)DO(3)*
D 547 (2738)	D 603 (2780)
R_b = 1.00	R_b = 1.00
RN**	
87	**95**
5β-P11α-DO(3)	5α-P11α-DO(3)
D 612 (2787)	D 623 (2794)
ΔDO(3) = 137	ΔDO(3) = 141
R_b = 0.900	R_b = 0.930
HY**	
98	**97**
5β-P11α(3)	5α-P11α(3)
D 447 (2650)	D 450 (2653)
R_b = 0.863	R_b = 0.864

Diagram 8. Synthesis of 5β-P11α(3) and 5α-P11α(3). The RD reduction of the (3)-steroids were 5β-P3α11α and 5α-P3β11α, respectively, identical to products obtained by a different method (*cf.* Diagram 6).

* For preparation of this compound *cf.* ref. 2, Diagram 11.

** This reaction was carried out with purified material extracted from TLC zone of R_b value indicated. Product concentration refers to material extracted from TLC zone of R_b value indicated.

A	B
5βP(11)DO(20)*	5αP(11)DO(20)*
D,N 572 (2757)	D,N 634 (2802)
R_b = 1.00	R_b = 1.00
RN**	
95	**90**
5βP11αDO(20)	5αP11αDO(20)
D 602 (2780)	D 620 (2792)
ΔDO(20) = 245	ΔDO(20) = 248
R_b = 0.893	R_b = 0.895
HY**	
95	**92**
5βP11α(20)	5αP11α(20)
D 343 (2535)	D 350 (2544)
R_b = 0.860	R_b = 0.870
RD	
58	**58**
5βP11α20β	5αP11α20β
D 526 (2721)	D 533 (2727)
R_b = 0.356	R_b = 0.352
+	+
34	**34**
5βP11α20α	5αP11α20α
D 476 (2677)	D 485 (2686)
R_b = 0.482	R_b = 0.455

Diagram 9. Synthesis of 5βP11α(20), 5αP11α(20), 5βP11α20β, 5αP11α20α, 5βP11α20α and 5αP11α20α.

* For preparation of this compound, *cf.* ref. 2, Diagram 11.

** This reaction was carried out with purified material extracted from TLC zone of R_b value indicated. Product concentration refers to material extracted from TLC zone of R_b value indicated.

ΔDO values as defined by footnote*** in the diagrams are given for DO derivatives. Last, the R_b value of products is given when TLC was used as a purification step; R_b is the migration distance relative to that of the dye Sudan blue taken as 1.00.

Tables II and III list the M_R values for the androstane and pregnane series, respectively.

TABLE II

M_R VALUES AND SOURCES OF M-STEROIDS OF THE ANDROSTANE SERIES

Steroid		M_R	*Source*
M	*Formula*		
I	5βA	1887	A 3000
II	5αA	1924	A 700
III	5βA3β	2175	A 3400
IV	5αA3α	2175	A 2150
V	5βA(3)	2184	Prepared; *cf.* ref. 1, Diagram 1 and 2
VI	5βA3α	2193	Prepared; *cf.* ref. 1, Diagram 2
VII	5αA(3)	2228	A 2650
VIII	Δ4A3β	2256	Calculated; from L_R Δ4A3β17β − G_R 17β*
IX	Δ5A3β	2269	A 8290
X	5αA3β	2279	A 2180
XI	Δ4A(3)	2305	Calculated; from L_R Δ4A17β(3) − G_R 17β* and L_R Δ4A(3,17) − G_R (17)**

* *Cf.* ref. 1, Table X.

** *Cf.* ref. 1, Table IX.

TABLE III

M_R VALUES AND SOURCES OF M-STEROIDS OF THE PREGNANE SERIES*

Steroid		M_R	*Sources*
M	*Formula*		
I	5βP	2113	P 5700
II	5αP	2150	P 1800
III	5βP3β	2402	Prepared; WK-5βP3β(20)
IV	5αP3α	2401	Calculated; M_R 5αA3α** + 226*** = 2401
V	5βP(3)	2412	Calculated; M_R 5βA(3)** + 226*** = 2412
VI	5βP3α	2421	P 7800
VII	5αP(3)	2453	P 4200
VIII	Δ4P3β	2483	Calculated; M_R Δ4A3β** + 226*** = 2483
IX	Δ5P3β	2497	Q 5350
X	5αP3β	2506	P 3450
XI	Δ4P(3)	2531	Calculated; M_R Δ4A(3)** + 226*** = 2531

* *Cf.* ref. 1, Table II, and ref. 2, Table IV.
** For M_R values, *cf.* ref. 1, Table I.
*** *Cf.* Ref. 1, eqn. 17.

Tables IV – X show the corrected retention times, t'_{NR}, the L_R, and G_R values, and the sources of steroids belonging, respectively, to groups A11α, A11α(17), A11α17β, P11α, P11α(17), P11α(20), P11α20β, and P11α20α. The G_R values were calculated from

$$G_R = L_R - M_R \qquad \text{(eqn. 9 in ref. 1)}$$

where M_R is taken from Table II or III.

TABLE IV

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP A11α

Steroid		t'_{NR}	L_R	G_R*	*Source*
M	*Formula*				
I	5βA11α	140	2146	259**	Prepared; *cf.* Table I
II	5αA11α	143	2155	231**	Prepared; *cf.* Table I
III	5βA3β11α	247	2393	218**	Calculated; L_R 5βP3β11α*** − ΔG_R§
IV	5αA3α11α	229	2360	185**	Prepared; *cf.* Table I
V	5βA11α(3)	298	2467	283**	Calculated; L_R 5βA11α(3,17)*** − ΔG_R§
VI	5βA3α11α	251	2399	206	Prepared; *cf.* Table I, and Diagram 2
VII	5αA11α(3)	296	2471	243**	Calculated; L_R 5αA11α(3,17)*** − ΔG_R§
VIII	Δ4A3β11α	287	2458	202	Calculated; L_R Δ4A3β11α17β*** − ΔG_R§
IX	Δ5A3β11α	295	2470	201	Prepared; *cf.* Diagram 3,B
X	5αA3β11α	300	2477	198	Prepared; *cf.* Table I and Diagram 3
XI	Δ4A11α(3)	367	2564	259**	Calculated; L_R Δ4A11α(3,17)*** − ΔG_R§

* Average G_R-normal = G_R A11α = 202.0.
** G_R-odd steroid.
*** For L_R value, *cf.* appropriate table.
§ For appropriate ΔG_R value, *cf.* Table XI.

TABLE V

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP A11α(17)

Steroid		t'_{NR}	L_R	G_R*	*Source*
M	*Formula*				
I	5βA11α(17)	235	2371	484**	Prepared; *cf.* Diagram 1,A
II	5αA11α(17)	241	2382	456**	Prepared; *cf.* Diagram 1,B
III	5βA3β11α(17)	417	2621	446**	Calculated; L_R 5βP3β11α(20)*** $-\Delta G_R$§
IV	5αA3α11α(17)	386	2586	411**	Prepared; *cf.* Diagram 2,B
V	5βA11α(3,17)	494	2693	509**	Prepared; *cf.* Diagram 4,A
VI	5βA3α11α(17)	422	2626	433	Prepared; *cf.* Diagram 2,A
VII	5αA11α(3,17)	498	2697	469**	Prepared: *cf.* Diagram 4,B
VIII	Δ4A3β11α(17)	488	2684	428	Calculated; L_R Δ4A3β11α17β*** $-\Delta G_R$§
IX	Δ5A3β11α(17)	496	2696	427	Prepared; *cf.* Diagram 3,B
X	5αA3β11α(17)	506	2704	425	Prepared; *cf.* Diagram 3,A
XI	Δ4A11α(3,17)	616	2790	485**	Prepared; *cf.* Diagram 4,C

* Average G_R-normal = G_R A11α(17) = 428.0.
** G_R-odd steroid.
*** For L_R value, *cf.* appropriate table.
§ For ΔG_R value, *cf.* Table XI.

TABLE VI

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP A11α17β

Steroid		t'_{NR}	L_R	G_R*	*Source*
M	*Formula*				
I	5βA11α17β	265	2423	536**	Prepared; *cf.* Table I and Diagram 1,A
II	5αA11α17β	273	2436	512**	Prepared; *cf.* Table I and Diagram 1,B
III	5βA3β11α17β	475	2677	502**	Calculated; L_R 5βP3β11α20β*** $-\Delta G_R$§
IV	5αA3α11α17β	441	2644	469**	Prepared; *cf.* Diagram 2,B
V	5βA11α17β(3)	561	2748	564**	Calculated; L_R 5βA11α(3,17)*** $+\Delta G_R$§
VI	5βA3α11α17β	467	2669	476**	Prepared; *cf.* Table I and Diagram 2,A
VII	5αA11α17β(3)	565	2752	524**	Calculated; L_R 5αA11α(3,17)*** $+\Delta G_R$§
VIII	Δ4A3β11α17β	548	2739	483	Prepared; *cf.* Diagram 4,C
IX	Δ5A3β11α17β	567	2753	484	Prepared; *cf.* Diagram 3,B
X	5αA3β11α17β	574	2759	480	Prepared; *cf.* Diagram 3,A
XI	Δ4A11α17β(3)	700	2845	540**	Calculated; L_R Δ4A11α(3,17)*** $+\Delta G_R$§

* Average G_R-normal = G_R A11α17β = 484.0.
** G_R-odd steroid.
*** For L_R value, *cf.* appropriate table.
§ For appropriate ΔG_R value, *cf.* Table XI.

In Tables II–IX, under Source, a capital letter followed by four digits is the catalogue No. of Steraloids (Pawling, N.Y., U.S.A.); SRC stands for Steroid Reference Collection (*cf.* Acknowledgements).

In Table XI, the G_R values shown in the forelast column are taken from Tables IV–X, footnote*. Table XI, last column, also shows ΣG_R values (*cf.* footnote ***) of multifunctional groups for comparison. ΔG_R values listed in Table XI were calculated for pairs of 11α-hydroxysteroids in all possible combinations from

$$\Delta G_R(a,b) = L_R(a) - L_R(b) \qquad \text{(eqn. 13 in ref. 1)}$$

with $L_R(a) > L_R(b)$.

TABLE VII

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P11α

Steroid		t'_{NR}	L_R	G_R*	*Source*
M	*Formula*				
I	5βP11α	211.5	2325	212**	Prepared; *cf.* Diagrams 5 and 7
II	5αP11α	215	2332	182**	Prepared; *cf.* Diagrams 5 and 7
III	5βP3β11α	374	2572	171**	Prepared; *cf.* Diagram 6
IV	5αP3α11α	346	2539	138**	Calculated; L_R 5αA3α11α*** + ΔG_R§
V	5βP11α(3)	447	2650	229**	Prepared; *cf.* Diagram 8
VI	5βP3α11α	380	2579	158	Prepared; *cf.* Diagram 6
VII	5αP11α(3)	450	2653	200**	Prepared; *cf.* Diagram 8
VIII	Δ4P3β11α	436	2640	159	Calculated; L_R Δ4P3β11α20β*** − ΔG_R§
IX	Δ5P3β11α	448	2651	154	Calculated; L_R Δ5P3β11α20β*** − ΔG_R§
X	5αP3β11α	452	2655	149	Prepared; *cf.* Diagram 6
XI	Δ4P11α(3)	556	2745	214**	Calculated; L_R Δ4P11α(3,20)*** − ΔG_R§

* Average G_R-normal = G_R P11α = 155.0.
** G_R-odd steroid.
*** For L_R value, *cf.* appropriate table.
§ For appropriate ΔG_R value, *cf.* Table XI.

TABLE VIII

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P11α(20)

Steroid		t'_{NR}	L_R	G_R*	*Source*
M	*Formula*				
I	5βP11α(20)	343	2535	422**	Prepared; *cf.* Diagram 9,A
II	5αP11α(20)	350	2544	394**	Prepared; *cf.* Diagram 9,B
III	5βP3β11α(20)	608.5	2784	382**	Prepared; *cf.* Diagram 6,A
IV	5αP3α11α(20)	561	2749	350**	Calculated; L_R 5αA3α11α(17)*** + ΔG_R§
V	5βP11α(3,20)	721	2858	446**	Prepared; *cf.* Diagram 7,A
VI	5βP3α11α(20)	617	2790	369	Prepared; *cf.* Diagram 6,B
VII	5αP11α(3,20)	732	2864	411**	Prepared; *cf.* Diagram 7,B; P 3650
VIII	Δ4P3β11α(20)	710	2851	368	Calculated; L_R Δ4P3β11α20β*** − ΔG_R§
IX	Δ5P3β11α(20)	728	2862	365	Calculated; L_R Δ5P3β11α20β*** − ΔG_R§
X	5αP3β11α(20)	741	2870	364	Prepared; *cf.* Diagram 6,C
XI	Δ4P11α(3,20)	908	2958	427**	SRC; prepared; *cf.* Diagram 7,C; Q 3240

* Average G_R-normal = G_R P11α(20) = 366.5.
** G_R-odd steroid.
*** For L_R value, *cf.* appropriate table.
§ For appropriate ΔG_R value, *cf.* Table XI.

Table XII shows L_R values of steroids of groups A11α, A11α(17) and A11α17β calculated from M-corresponding members of groups P11α, P11α20, P11α20β and P11α20α by using

$$L_R(b) = L_R(a) - \Delta G_R(a,b) \qquad \text{(eqn. 15 in ref. 1)}$$

with $\Delta G_R(a,b)$ values taken from Table XI.

TABLE IX

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P11α20β

Steroid		t'_{NR}	L_R	G_R*	*Source*
M	*Formula*				
I	5βP11α20β	526	2721	608**	Prepared; *cf.* Diagram 9,A
II	5αP11α20β	533	2727	577**	Prepared; *cf.* Diagram 9,B
III	5βP3β11α20β	930	2968	564**	Prepared; *cf.* Diagram 6,A
IV	5αP3α11α20β	859	2935	534**	Calculated; L_R 5αA3α11α17β*** + ΔG_R§
V	5βP11α20β(3)	1097	3040	626**	Calculated; L_R 5βP11α(3,20)*** + ΔG_R§
VI	5βP3α11α20β	935	2970	549	Prepared; *cf.* Diagrams 6,B and 7,A
VII	5αP11α20β(3)	1113	3046	593**	Calculated; L_R 5αP11α(3,20)*** + ΔG_R§
VIII	Δ4P3β11α20β	1075	3033	550	Prepared; *cf.* Diagram 7,C
IX	Δ5P3β11α20β	1107	3044	547	Calculated; L_R Δ5A3β11α17β*** + ΔG_R§
X	5αP3β11α20β	1120	3049	543	Prepared; *cf.* Diagrams 6,C and 7,B
XI	Δ4P11α20β(3)	1380	3140	609**	Calculated; L_R Δ4P11α(3,20)*** + ΔG_R§

* Average G_R-normal = G_R P11α20β = 547.0.
** G_R-odd steroid.
*** For L_R value, *cf.* appropriate table.
§ For appropriate ΔG_R value, *cf.* Table XI.

TABLE X

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P11α20α

Steroid		t'_{NR}	L_R	G_R*	*Source*
M	*Formula*				
I	5βP11α20α	476	2677	564**	Prepared; *cf.* Diagram 9,A
II	5αP11α20α	485	2686	536**	Prepared; *cf.* Diagram 9,B
III	5βP3β11α20α	844	2926	521**	Prepared; *cf.* Diagram 6,A
IV	5αP3α11α20α	782	2893	492**	Calculated; L_R 5αP3α11α20β*** − ΔG_R§
V	5βP11α20α(3)	996	2998	586**	Calculated; L_R 5βP11α20β(3)*** − ΔG_R§
VI	5βP3α11α20α	852	2930	509	Prepared; *cf.* Diagrams 6,B and 7,A
VII	5αP11α20α(3)	1010	3004	551**	Calculated; L_R 5αP11α20β(3)*** − ΔG_R§
VIII	Δ4P3β11α20α	976	2989	506	Prepared; *cf.* Diagram 7,C
IX	Δ5P3β11α20α	1005	3002	505	Calculated; L_R Δ5P3β11α20β*** − ΔG_R§
X	5αP3β11α20α	1022	3009	503	Prepared; *cf.* Diagrams 6,C and 7,B
XI	Δ4P11α20α(3)	1252	3098	567**	Calculated; L_R Δ4P11α20β(3)*** − ΔG_R§

* Average G_R-normal = G_R P11α20α = 505.7.
** G_R-odd steroid.
*** For L_R value, *cf.* appropriate table.
§ For appropriate ΔG_R value, *cf.* Table XI.

DISCUSSION

Reactions

At the onset of the present investigation, the only 11α-hydroxysteroid standards available from commercial and other sources were 5αP11α(3,20) and Δ4P11α-(3,20) (*cf.* Table VIII). The RN reduction of 11-ketosteroids, effects of this reaction on other functional groups and structural features, and effects of other reactions on functional groups in the presence of 11α were studied more extensively than has been hitherto reported.

TABLE XI

ΔG_R, G_R AND ΣG_R VALUES

Group	ΔG_R*							G_R**	ΣG_R***
	A11α	*A11α(17)*	*A11α17β*	*P11α*	*P11α(20)*	*P11α20β*	*P11α20α*		
A11α	—	226	281	179	391	573	532	202.0	—
A11α(17)	226	—	55	47	165	347	305	428.0	465.2
A11α17β	281	55	—	100	110	291	248	484.0	549.0
P11α	179	47	100	—	213	393	352	155.0	—
P11α(20)	391	165	110	213	—	182	140	366.5	389.0
P11α20β	573	347	291	393	182	—	42	547.5	508.5
P11α20α	532	305	248	352	140	42	—	505.7	535.0

* ΔG_R value for a group combination is average of ΔG_R values for M-corresponding pairs of steroids, *i.e.* the difference of L_R values $\Delta G_R = L_R(a) - L_R(b)$ with $L_R(a) > L_R(b)$.

** G_R values are G_R-normal values shown in footnote * in Tables IV–X.

*** ΣG_R is sum of G_R-normal values of groups featuring one component functional group only, *e.g.* ΣG_R A11α(17) = G_R A11α + G_R A(17).

Note: G_R A(17) = 262.7; G_R A17β = 346.5 (*cf.* ref. 1, Table XII); G_R P(20) = 234; G_R P20β = 353.5; G_R P20α = 380 (*cf.* ref. 2, Table XVI).

RD. Reduction by sodium borohydride of keto groups proceeded in the presence of 11α in a way very similar to that observed in the presence of (11) or 11β. Thus, (3) was converted to 3β except 5β(3) which yielded 5β3α; (17) yielded 17β. In all cases, the yield of the minor stereoisomer was extremely small. RD reduction of 11α(20)-steroids yielded two hydroxysteroids, that with the largest t'_{NR} value being in the highest proportion (66:34) (*cf.* Diagrams 6, 7 and 9). From previous observations on the RD reduction of (20) (ref. 2, Table I), the major isomer should be the 11α20β-compound. This was confirmed by the fact that this compound migrated on TLC plates in our system very distinctly behind the minor isomer; it was therefore the more polar, as previously observed for 20β-compounds[2]. This assignment was further confirmed by the results of RN reduction (see below).

RN. Because nascent hydrogen generated at the sodium–ethanol interface is rapidly converted to inactive molecular hydrogen, and because bubbles of hydrogen covering the surface hinder the access of ketone molecules to the reaction site, this reaction is rather inefficient as regards the sodium used. From the volume of acetic acid used to neutralize the reaction mixture, about 1000 times the stoichiometric amount of sodium was needed for 85–95% conversion of ketones. Under present conditions, this is still a small amount of sodium. As an excess considerably over the above requirement did not produce adverse effects, the procedure was simplified by using in all cases involving from 0 to 1 mg of ketone, that amount of sodium which was sufficient for 1 mg. Under these conditions, most of the material balance, *i.e.* from 5 to 15%, was still unconverted ketone easily separable by TLC. The much higher polarity (slow migration) of 11α-hydroxysteroids as compared with (11)- and 11β-hydroxysteroids always ensured a sharp separation from other products by TLC. Compare, for example, the R_b values of homologous 11β- and 11α-pregnane derivatives shown in ref. 2 and Diagrams 5–9 in this article, respectively. TLC bands of 11α-hydroxysteroids were clearly revealed on plates sprayed with phosphomolybdic acid, even in trace amounts.

TABLE XII

L_R VALUES OF STEROIDS OF GENERAL FORMULA MA11α, MA11α(17) AND MA11α17β CALCULATED FROM THE L_R VALUES OF M-CORRESPONDING STEROIDS OF RELATED GROUPS P11α, P11α(20), P11α20β AND P11α20α WITH APPROPRIATE ΔG_R VALUES TAKEN FROM TABLE XI

M	L_R *MA11α*					L_R *MA11α(17)*					L_R *MA11α17β*				
	P11α	*P11α(20)*	*P11α20β*	*P11α20α*	*Found***	*P11α*	*P11α(20)*	*P11α20β*	*P11α20α*	*Found***	*P11α*	*P11α(20)*	*P11α20β*	*P11α20α*	*Found***
5βA	2146	2144	2148	2145	2146 (2146)	2372	2370	2374	2372	2371 (2372)	2425	2425	2430	2429	2423 (2427)
5αA	2153	2153	2154	2154	2155 (2154)	2379	2379	2380	2384	2382 (2383)	2432	2434	2436	2438	2436 (2435)
5βA3β	2393*	2393	2395	2394	2393 (2394)	2619	2621*	2621	2621	2621 (2621)	2672	2674	2677*	2678	2677 (2675)
5αA3α	2360*	2359	2362	2361	2360 (2361)	2586	2586*	2588	2588	2586 (2586)	2639	2639	2644*	2645	2644 (2642)
5βA(3)	2467	2467	2471	2466	2471 (2468)	2697	2693	2693	2693	2693 (2694)	2750	2748	2749	2750	2748 (2749)
5βA3α	2400	2399	2397	2398	2399 (2399)	2626	2625	2623	2625	2626 (2625)	2679	2680	2679	2682	2669*** (2680)
5αA(3)	2474	2473	2473	2472	2471 (2473)	2700	2699	2699	2699	2697 (2699)	2753	2754	2755	2756	2752 (2754)
Δ4A3β	2461	2460	2460	2457	2458 (2460)	2687	2686	2686	2684	2684 (2686)	2740	2741	2742	2741	2739 (2741)
Δ5A3β	2472	2471	2471*	2470	2470 (2471)	2698	2698	2697*	2697	2696 (2698)	2751	2752	2753*	2754	2753 (2753)
5αA3β	2476	2479	2476	2477	2477 (2477)	2702	2705	2702	2704	2704 (2704)	2755	2760	2758	2761	2759 (2759)
Δ4A(3)	2568	2567	2567	2566	2564 (2567)	2794	2793	2793	2793	2790 (2793)	2845	2848	2849	2850	2845 (2848)

* Good agreement for this value was to be expected (*cf.* text).

** Quantity in brackets under *Found* is average of calculated values.

*** Disagreement between found and calculated values is disctssed tn the text.

Table I shows that the reduction of 11-ketosteroids of the androstane series proceded uneventfully with the exception of Δ4A compounds. The reduction of (3) and (17) proceded in a manner similar to that observed in RD reduction including the absence of effect on Δ5A. Reduction of Δ4A(3)-, mainly to 5αA3β-, was observed. Some reduction of Δ4A3β- did occur; it was complicated by the loss of 3β or 17β, or both, resulting in the appearance of several early peaks in GLC chromatograms. However, DO derivatization of Δ4A(3)- afforded complete protection to the Δ4A double bond (*cf.* Diagram 4,C).

A very similar behaviour was observed with corresponding ketones of the pregnane series, including the protective effect of DO derivatization of (3) in Δ4P(3)- (*cf.* Diagram 7,C).

RN reduction of (20) led to two stereoisomeric hydroxysteroids whose retention times matched that of 20α- and 20β-isomers obtained by RD reduction. However, the 20α:20β ratio now was 60:40, *i.e.* it was reversed, as indeed observed by Kirk in the reduction of 5αP(20) by sodium–ethanol[5]. RN reductions therefore confirmed assignments of 20α- and 20β-isomers described above.

DO. Although DO derivatization was not carried out with 11α-hydroxysteroids, properties of 11αDO derivatives were observed and ΔDO values were recorded. It was obvious that ΔDO(3), ΔDO(3,17), ΔDO(20) and ΔDO(3,20) values (*cf.* Diagrams 1–9) were as predictable as their (11)ΔDO and 11$\beta\Delta$DO counterparts[1,2], and as readily distinguishable from each other. Hence, ΔDO values afford a means of characterizing 11α-hydroxysteroids and the presence of various other functional groups at positions 3(A or P), 17(A), and 20(P). Furthermore, as corresponding (11)ΔDO, 11$\beta\Delta$DO, and 11$\alpha\Delta$DO values are numerically distinct, and as 11β[1,2] and 11α (*cf.* below) are readily converted by chromium trioxide oxidation to (11), RN and RD reduction of DO derivatives can be used independently for the characterization of 11-substituted steroids.

The ΔDO(3,20) value corresponding to Δ4P11αDO(3,20) (Diagram 7,C) was much lower than expected from that of homologous steroids (Diagram 7,A and B). Whatever the nature of the DO derivative[6], the hydrolytic product was definitely identified as Δ4P11α(3,20) by its t'_{NR} value and RD reduction products. The similar case of Δ4P11βDO(3,20) has been discussed[2].

The DO derivatives of 11α-hydroxysteroids were very easily hydrolyzed by acid. Their isolation after RN reduction of (11)DO derivatives, required careful neutralization with acetic acid. Use of hydrochloric acid led directly to the free steroid (*cf.* Diagram 4).

WK. The removal of keto groups by the Wolff–Kishner reaction proceded uneventfully in the presence of 11α (*cf.* Diagrams 2, 3, 6, 7) except with Δ4P11α(3,20) (*cf.* Diagram 7,C) where it was complicated by partial reduction of Δ4P to 5αP and 5βP and resulted in the appearance of several early, closely spaced peaks in GLC chromatograms.

OX. One hour of oxidation by chromium trioxide sufficed to convert 11α quantitatively to (11). This reaction was used routinely to confirm the identity of 11α-hydroxysteroids by converting them to known ketones.

TMS. Derivatization of 11α was often incomplete under conditions previously described[1], but always complete at 32–35° (*cf.* above). The retention-time shifts induced by TMS derivatization are exemplified in the following data where the shift

shown in brackets is expressed in L_R units preceded by the appropriate sign: 5βA11α [+46], 5αA11α [+13], 5βA3α11α [+25]; and 5αP11α [−32], 5βP3β11α [−36], 5βP3α11α [−34], 5αP3β11α [+44].

Obviously, the shift was unpredictable in direction and extent.

HY. The hydrolyses of TMS and DO derivatives were complete under standard conditions[1].

In Diagrams 1–9, the data clearly demonstrate the similar behaviour of homologous steroids in RN reductions and of the products in subsequent steps.

G_R and ΔG_R data

Groups of 11α-hydroxysteroids of the androstane and pregnane series in Tables IV–X display the same pattern of G_R-odd steroids except group A11α17β (Table VI) where 5βA3α11α17β appears as an extra G_R-odd member. While G_R-odd steroids are most numerous in the general pattern, the G_R-normal status assigned to the 5β3α-, Δ4 3β-, Δ5 3β-, and 5α3β- members of each group is justified by all four having approximately the same G_R values. In contrast, members to which G_R-odd status was assigned have G_R values widely scattered about the G_R-normal value, all but one (5α3α) being higher than this value. In contrast, G_R-oddity for groups of 11-keto- and 11β-hydroxysteroids previously reported for the androstane[1] and pregnane[2] series was always negative. In fact, the only similarity between the G_R patterns of these steroids and that of 11α-hydroxysteroids is the G_R-normalcy of Δ5 3β- and 5α3β-members.

A comparison of G_R-normal values for multifunctional steroids with the corresponding ΣG_R values listed in Table XI again demonstrates that G_R values for such groups cannot be predicted from the G_R-normal values of component functional groups[1,2].

Table XII shows 132 L_R values calculated through the ΔG_R method using ΔG_R values listed in Table XI. The perfect fit of calculated L_R values indicated by * should be disregarded as the corresponding "*Found*" values taken from Tables IV – VI (5βA3β-), or the values from which the values were calculated, Table X (5αP3β-, and Δ5P3β-), were themselves obtained by the ΔG_R method. On the other hand, the poor fit of calculated L_R values for 5βA3α11α17β resulted from this compound having abnormal, excessive oddity just as its 5βA3α11β17β counterpart[1]. Of the remaining calculated L_R values, 90% fell within 2 L_R units and 98% within 3 L_R units of observed values; only two values had an error in excess of 3 units, yet less than 1% of the retention time. Errors on averages of calculated values never exceeded 3 L_R units.

These results bring strong additional support to the general validity of a rule previously discussed[2], *i.e.* that G_R-oddity is quantitatively the same in M-corresponding members of steroid groups which feature the same oddity-inducing functional group. These results again demonstrate the versatility and reliability of L_R value calculations based on this key principle[1,2].

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