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

II. POSITIONS 3, 11, AND 20 IN THE PREGNANE SERIES*

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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 (WK), their conversion to hydroxyl groups by $NaBH_4$ (RD), and to dioxolone derivatives by ethylene glycol (DO); the conversion of hydroxyl to keto groups by CrO_3 (OX), and to TMS ethers by hexamethyldisilazane; the hydrolysis of dioxolone and TMS derivatives by H^+ (HY). GLC chromatograms of reaction mixtures of single and multistep reactions readily provide information on effects on functional groups at positions 3, 11, and 20 in the 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 computation methods is demonstrated.

INTRODUCTION

The use of two internal standards in the gas–liquid chromatographic (GLC) analysis of steroids has afforded a simple means of determining retention times under highly reproducible conditions precisely correlated to nominal retention times A and B of the standards[1,2]. By applying corrections for small fluctuations of temperature and carrier gas velocity based on observed retention times A' and B' of the standards (*cf.* eqns. 1–5 in ref. 2), normalized retention times, t'_{NR}, were consistently reproduced for all steroids throughout the long life of the columns[2]. The number of steroids that could conceivably correspond to an observed retention time thus became very small,

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and consequently, positive identification of peaks through independent discriminatory methods was considerably simplified. Precise retention time measurements also permitted a reliable comparison of data from which the rules relating t'_{NR} to steroid structure were established. The precision of retention times computed on the basis of these rules was unequivocally demonstrated[2].

It is the purpose of the present series to show that the above rules which take into consideration the various structural features commonly found in naturally occurring steroids can be applied generally and used effectively to identify these compounds (*cf.* Discussion).

In Part I[2], the logarithmic expression of t'_{NR} defined as $L_R = 10^3 \times \log t'_{NR}$ (eqn. 6 in ref. 2) was obtained for 99 steroids of the androstane series characterized by any of the following functional groups alone or in all possible combinations: 3-, 11- and 17-keto, 3α-, 3β-, 11β- and 17β-hydroxy. Otherwise unobtainable steroids were systematically synthesized by the application of classical reactions using procedures adapted to amounts from 0 to 1 mg, and to the GLC observation of reaction mixtures. This work not only afforded required t'_{NR} values but also numerous observations on the course of the classical reactions with groups at positions 3, 11, and 17, which could be used in identification[2].

In the present article, data on the application of the same synthetic procedures to groups at positions 3, 11, and 20 of the pregnane molecule are described in Tables I–III and Diagrams 1–11. Observations on the course of the reactions with these groups are presented and discussed. Following the pattern previously adopted, the 121 steroids relevant to this study were classified so as to emphasize the M- and G-features, *i.e.*, those which affect the structure of ring A and that of the rest of the molecule, respectively[2]. The resulting Tables V–XV list these steroids according to M-features for each of the following 11 groups: P(11), P(11β), P(11,20), P11β(20), P20β(11), P20α(11), P11β20β, P11β20α, P(20), P20β, and P20α. Among other data, these tables show the G_R values (contribution to L_R of G-features) derived from $L_R = M_R + G_R$ (eqn. 8 in ref. 2), where M_R, the contribution to L_R of M-features, is the logarithmic expression $M_R = 10^3 \times \log t'_{MR}$ (eqn. 7 in ref. 2) of retention time, t'_{MR}, of the corresponding M-steroid, *i.e.*, that with M-features only[2]. M_R and t'_{MR} values for the M-steroids of the pregnane series are listed in Table IV.

The data were analyzed (*cf. GLC data analysis* in ref. 2) to obtain the patterns of G_R-normal and G_R-odd steroids, and the ΔG_R values of related groups (Table XVI). Relatedness[2] with groups of the androstane series[2] were also established (Table XVI) and the resulting ΔG_R values were used to compute the L_R values for steroids relevant to the present article from the L_R values of M-corresponding steroids described in Part I[2]. Errors obtained by comparison of computed with observed L_R values are shown in Table XVII.

Symbols and abbreviations, including shorthand formulae of steroids used in this series have been defined[2].

EXPERIMENTAL

The GLC techniques and the preparative procedures relevant to this article have been described[2]. The classical reactions used included: RD, reduction of keto groups by $NaBH_4$; WK, reductive removal of keto groups through the Wolff–Kishner

reaction; DO, conversion of keto groups to dioxolone (ketal) derivatives; OX, CrO_3 oxidation of alcohols to ketones; TMS, conversion of OH groups to trimethylsilyl ethers; HY, acid hydrolysis of dioxolone or TMS derivatives.

The procedures were applied as described[2] except in two cases, where an increase in the yield of desired products required the following modifications (*cf.* Discussion):

(a) The DO derivatization of 20-ketones was performed with only 0.5 mg of paratoluenesulfonic acid by extending the reaction time from 5 to 7.5 h with toluene addition every hour.

(b) The 30-min RD reduction of 5βP(11,20) and 5αP(11,20) was performed with 2 mg of $NaBH_4$ only (*cf.* Table II).

Thin-layer chromatography (TLC), performed as described in ref. 3, was used extensively to purify reaction products (*cf.* Discussion): When this was the case a test plate obtained with part of the reaction mixture was sprayed[3,4] to locate the major products. The resulting information was transferred to an unsprayed plate from which the bands were eluted[3], and the gas–liquid chromatograms before and after purification were compared. The inclusion of pilot dyes[3] in all solutions submitted to TLC helped considerably in locating bands on the unsprayed plates[3] (*cf.* Discussion).

Data

In Tables I–III and Diagrams 1–11, describing products of reactions or reaction sequences, data are usually restricted to the main products. Appropriate symbols in bold type at the right, and occasionally in the middle of the diagrams indicate the reactions involved. The percentage of product(s) in each reaction mixture is indicated in bold print above the relevant formula; this is followed by the t'_{NR} value (in 10^{-2} min) preceded by D or N to indicate whether the mixture was submitted to TMS derivatization or not.

When the DO reaction is involved, the L_R value of the initial steroid and that of its ketal derivative, both as TMS derivatives, and the difference ΔDO of these values are given. Finally, when TLC was used as a purification step, the R_b value of the compound, *i.e.*, its relative migration distance *vs.* Sudan blue[3,4], is shown.

In Tables IV–XV, listing t'_{NR}, L_R, M_R, and G_R values, the sources of steroids are indicated as follows: A capital letter followed by digits is the catalogue number of Steraloids Inc.*, where the compound originated; PREP denotes a steroid synthesized in this laboratory by the method indicated; SRC refers to a gift from the Steroid Reference Collection**; COMP denotes an L_R value computed through the method indicated.

DISCUSSION

Steroid preparation

In multistep preparations, the number of unwanted products tended to increase in successive steps due to the accumulation of compounds derived from by-products. As shown below, the DO reaction often used in preparations gave rise to

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an unusually high proportion of by-products. This, together with products and by-products from up to 10% impurities in the starting materials, made intermediate TLC purification steps desirable in many cases. Effective TLC purification often required the removal and elution of closely-spaced bands while limiting the material thus removed as much as possible to the width of such bands. Since this operation was performed with TLC plates that had not been sprayed to reveal the band positions so as to avoid contamination or alteration of products, the precise location of bands was obtained from sprayed test plates in relation to a number of pilot dyes included in all test solutions[3]. Although this procedure was rather wasteful of material and although complete purification was seldom obtained, the purity of main products and the clarity of relevant GLC chromatograms were much improved through its application.

In Tables I–III and Diagrams 1–11, data concerning the present preparations were limited to main products, to make apparent the remarkable similarity in behaviour of homologous compounds undergoing the same reactions. Observations on the course of these reactions with various functional groups can be summarized as follows.

RD. Under the conditions used[2], both (3) and (20) were completely reduced within 30 min, while the complete reduction of (11) required 2 h (Tables I and II). The results are symbolized as follows:

$5\alpha(3) \rightarrow 5\alpha 3\beta$; $5\beta(3) \rightarrow 5\beta 3\alpha$; $\Delta 4(3) \rightarrow \Delta 43\beta$; $(11) \rightarrow 11\beta$; $(20) \rightarrow 20\alpha + 20\beta$.

Reduction of (3) and (11) essentially yielded a single stereoisomer, as observed with (3), (11), and (17) in the androstane series[2].

The proportion of 20α isomer from the reduction of (20), while always lowest, was higher with a functional group present at position 11 (Table I). The proportion

TABLE I

REDUCTION BY $NaBH_4$ (2 h) OF 20-KETONES*

*Starting material***	*Source*	*Percentage of main products****		*20α/20β*
		20α	*20β*	
5βP3β(20)	P8180	15	85	15/85
5βP3α(20)	P8150	16	84	16/84
5αP3β(20)	P3830	14	86	14/86
				Av. 15/85
5αP3β11β(20)	SRC	28	72	28/72
5βP(11,20)	*cf.* Diagram 9A	25	75	25/75
5αP(11,20)	*cf.* Diagram 9B	25	75	25/75
5βP3β(11,20)	P7900	27	73	27/73
5βP(3,11,20)	Q500	30	70	30/70
5βP3α(11,20)	P7850	25	75	25/75
5αP(3,11,20)	P5630	25	75	25/75
5αP3β(11,20)	P3600	27	73	27/73
Δ4P(3,11,20)	Q4160	31	69	31/69
				Av. 28/72

* Under these conditions (11) is converted to 11β.

** GLC properties of starting materials: P(20) compounds, *cf.* Table XIII; P(11,20) compounds, *cf.* Table VII.

*** GLC properties of 20β compounds, *cf.* Table XIV; for 20α compounds, *cf.* Table XV; for 11β20β and 11β20α compounds, *cf.* Tables XI and XII, respectively.

TABLE II

REDUCTION BY $NaBH_4$ (30 min) OF 11,20-KETONES

Starting material[*]	*Concentrations, t'_{NR} and R_b values of main products*[***,§§]				*11β20α/11β20β*[§§§]
	20α(11)	*20β(11)*	*11β20α*	*11β20β*	
5βP(11,20)[**]	13 D 426 R_b = 0.770	37 D 408 R_b = 0.740	12 D 499 R_b = 0.700	38 D 442 R_b = 0.650	24/76
5αP(11,20)[**]	14 D 468 R_b = 0.770	37 D 457 R_b = 0.750	11 D 556 R_b = 0.710	38 D 501 R_b = 0.664	22/78
5βP3β(11,20)	12 D 850 R_b = 0.317	23 D 816 R_b = 0.257	13 D 981 R_b = 0.215	52 D 874 R_b = 0.145	20/80
5βP(3,11,20)[§]	12 D 845 R_b = 0.220	36 D 805 R_b = 195	10 D 985 R_b = 0.171	43 D 887 R_b = 0.124	19/81
5βP3α(11,20)	16 D 845 R_b = 0.220	35 D 805 R_b = 0.195	9 D 985 R_b = 0.171	40 D 887 R_b = 0.124	18/82
5αP(3,11,20)[§]	13 D 1068 R_b = 0.298	34 D 1048 R_b = 0.248	11 D 1278 R_b = 0.179	42 D 1158 R_b = 0.120	21/79
5αP3β(11,20)	14 D 1068 R_b = 0.298	31 D 1048 R_b = 0.248	11 D 1278 R_b = 0.179	44 D 1158 R_b = 0.120	20/80
Δ4P(3,11,20)[§]	19 D 973 R_b = 0.338	32 D 962 R_b = 0.287	13 D 1171 R_b = 0.261	36 D 1060 R_b = 0.117	26/74

[*] For GLC data and origin of 11,20 ketones, *cf.* Table VII.

[**] With only 2 mg of $NaBH_4$ (*cf.* text).

[***] Concentrations (%) rounded up to the nearest unit.

[§] 5βP(3), 5αP(3), and Δ4P(3) ketones yield 5βP3α, 5αP3β, and Δ4P3β products, respectively.

[§§] GLC properties of 20β(11), 20α(11), 11β20β, and 11β20α products are listed in Tables IX, X, XI, and XII, respectively.

[§§§] The ratio of 11β20α to 11β20β products averaged to 21/79 (*cf.* text).

of 11β20α *vs.* 11β20β product was lower after 30 min (Table II) than after 2 h (Table I). This indicated that the reduction of (11) was slower in the 20α(11) than in the 20β(11) isomer.

Unexpectedly, the reduction of 5αP(11,20) and 5βP(11,20) (*cf.* Table II) was almost complete in 30 min with 10 mg of $NaBH_4$[2]; reasonable yields of 20α(11) and 20β(11) compounds were obtained, however, when only 2 mg of reagent were used (*cf.* above).

In GLC chromatograms of the reduction mixtures, the relatively minor 20α(11) peak was in all cases overlapped by that of the 20β(11) and 11β20β products. The retention time of 20α compounds was always higher than that of the corresponding 20β.

TABLE III

GLC DATA* ON THE PREPARATION OF 5βP3β(11), 5βP(3,11) AND 5βP3β11β (SEQUENCE A), 5βP3α(11) AND 5βP3α11β (SEQUENCE B); 5αP3β(11), 5αP(3,11), AND 5αP3β11β (SEQUENCE C) BY THE METHOD EXEMPLIFIED IN DIAGRAM 6

Sequence	*Starting material*	*Source*	*Concentrations, t'_{NR} and L_R values of main products*				
			WK step		*RD (2 h) step***	*OX step*	
A	5βP3β(11,20) D 598 L_R = 2777	P7900	5βP3β(11) 48 D 366 L_R = 2563	5βP3β11β 45 D 414 L_R = 2617	5βP3β11β 94 D 414 L_R = 2617	5βP(3,11) 82 D,N 344 L_R = 2536	5βP3β(11) 12 D 366 L_R = 2563
B	5βP3α(11,20) D 598 L_R = 2776	P7850	5βP3α(11) 36 D 365 L_R = 2562	5βP3α11β 56 D 419 L_R = 2622	5βP3α11β 92 D 419 L_R = 2622	5βP(3,11) 70 D 344 L_R = 2536	5βP3α(11) 20 D 365 L_R = 2562
C	5αP3β(11,20) D 756 L_R = 2878	P3600	5αP3β(11) 35 D 460 L_R = 2663	5αP3β11β 54 D 540 L_R = 2732	5αP3β11β 92 D 540 L_R = 2732	5αP(3,11) 80 D 391 L_R = 2592	5αP3β(11) 10 D 460 L_R = 2663

* Limited to main products.

** The RD step yields mixtures including a high concentration of 11β product; direct oxidation of WK mixtures led to results very similar to those shown in the last two columns.

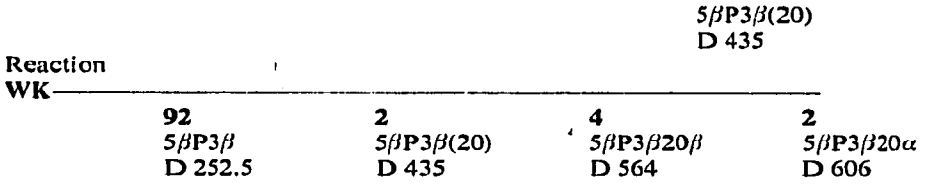

Diagram 1. Synthesis of 5βP3β.

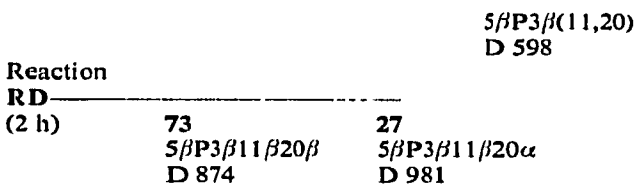

Diagram 2. Synthesis of 5βP3β11β20β and 5βP3β11β20α. Data relevant to the complete reduction of other (11,20) compounds are listed in Table I.

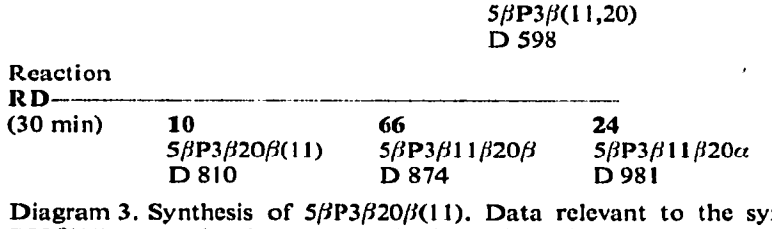

Diagram 3. Synthesis of 5βP3β20β(11). Data relevant to the synthesis of other members of the P20β(11) group by the same method are listed in Table II.

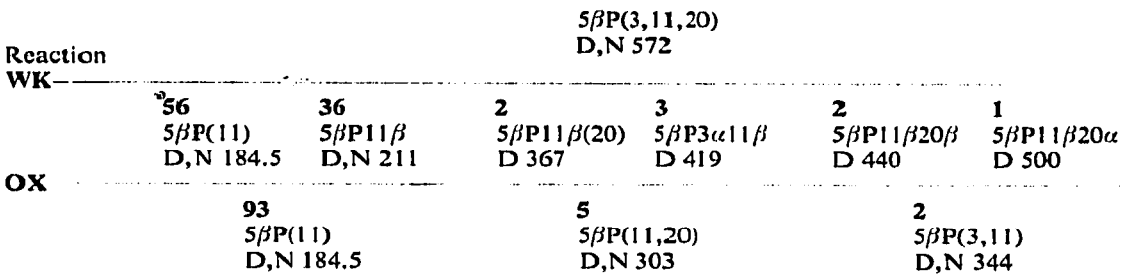

Diagram 4. Synthesis of 5βP(11) and 5βP11β.

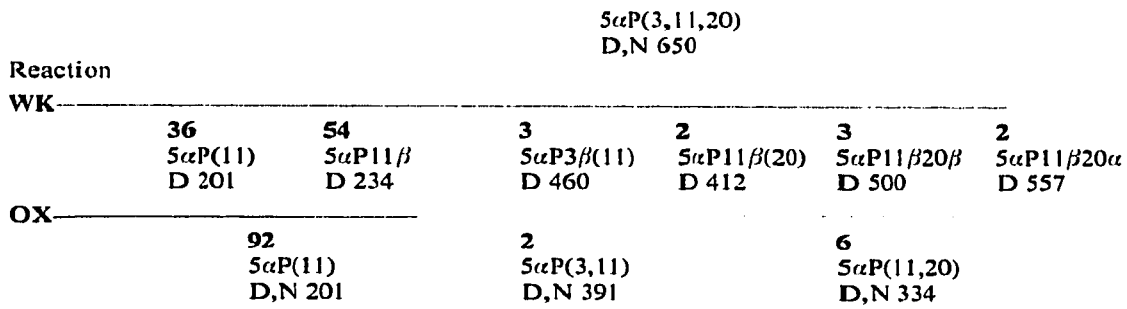

Diagram 5. Synthesis of 5αP(11) and 5αP11β.

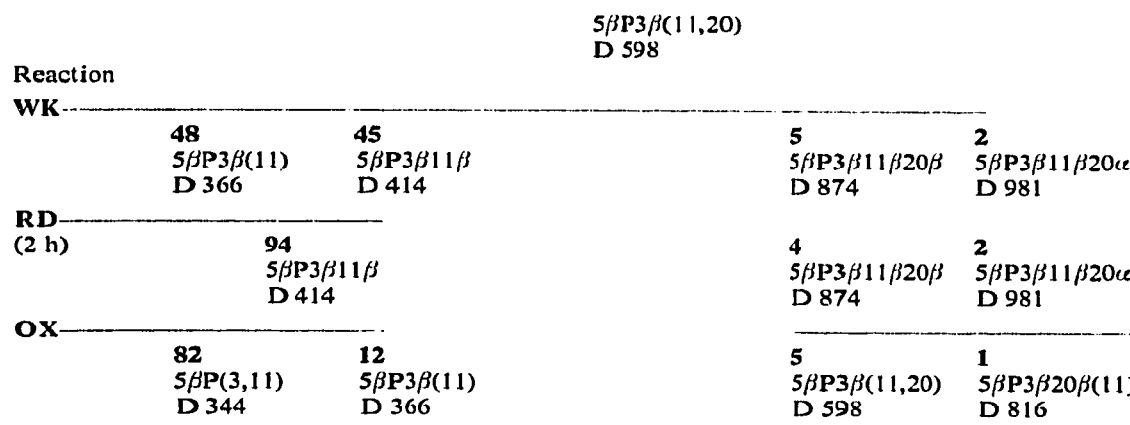

Diagram 6. Synthesis of 5βP3β(11), 5βP3β11β and 5βP(3,11).

As shown by R_b values, isolation of the 30-min reduction products (Table II) was made possible by the faster migration (lower polarity) of incompletely reduced products, and by the faster migration of the 20α isomer of both incompletely and completely reduced products.

There was evidence of some reduction of Δ4(3) to 5α3β and 5β3α. However, the Δ4 and Δ5 double bonds in Δ43β and Δ53β were unaffected.

Reaction	A 5β P3β(11,20) D 598 (2777)	B 5β P3α(11,20) D 598 (2776)	C 5α P3β(11,20) D 756 (2878)
DO	**93** 5β P3β(11)DO(20) D 1152 (3061) $\Delta DO(20) = 284$ $R_h = 0.700$	**95** 5β P3α(11)DO(20) D 1132 (3053) $\Delta DO(20) = 277$ $R_h = 0.700$	**80** 5α P3β(11)DO(20) D 1446 (3160) $\Delta DO(20) = 282$ $R_h = 0.700$
RD (2 h)	**94** 5β P3β11βDO(20) D 1293 (3112) $\Delta DO(20) = 244$ $R_h = 0.950$	**95** 5β P3α11βDO(20) D 1278 (3107) $\Delta DO(20) = 246$ $R_h = 0.950$	**85** 5α P3β11βDO(20) D 1690 (3228) $\Delta DO(20) = 256$ $R_h = 0.940$
HY	**90** 5β P3β11β(20) D 726 (2861) $R_h = 0.455$	**87** 5β P3α11β(20) D 723 (2859) $R_h = 0.437$	**75** 5α P3β11β(20) D 938 (2972) $R_h = 0.358$

Diagram 7. Synthesis of (A) 5β P3β11β(20), (B) 5β P3α11β(20) and (C) 5α P3β11β(20) by application of the reaction sequence DO, RD, HY to the corresponding (11,20) compounds. The percentage of the main product in each reaction mixture, and the R_h values (TLC) at the DO and RD steps are given (*cf.* text); $\Delta DO(20)$ is the difference between the L_R value of the compound and that of the ketalized compound, both as TMS derivatives (*cf.* text).

Reaction	A 5β P(3,11,20) D 572 (2758)	B 5α P(3,11,20) D 650 (2813)	C Δ14P(3,11,20) D 740 (2869)
DO	**93** 5β P(11)DO(3,20) D 1740 (3240) $\Delta DO(3,20) = 482$	**95** 5α P(11)DO(3,20) D 1926 (3284) $\Delta DO(3,20) = 471$	**75** Δ14P(11)DO(3,20)? D 1858 (3269) $\Delta DO(3,20) = 400$
RD (2 h)	**95** 5β P11βDO(3,20) D,N 2008 (3302) $\Delta DO(3,20) = 440$	**92** 5α P11βDO(3,20) D,N 2260 (3354) $\Delta DO(3,20) = 434$	**80** Δ14P11βDO(3,20)? D,N 2194 (3341) $\Delta DO(3,20) = 348$
HY	**90** 5β P11β(3,20) D,N 728 (2862) $R_h = 0.800$	**87** 5α P11β(3,20) D,N 832 (2920) $R_h = 0.816$	**75** Δ14P11β(3,20) D,N 984 (2993) $R_h = 0.715$

Diagram 8. Synthesis of (A) 5β P11β(3,20), (B) 5α P11β(3,20) and (C) Δ14P11β(3,20) by application of the reaction sequence DO, RD, HY to the corresponding (11,20) compounds. The percentage of the main product in each reaction mixture is given; $\Delta DO(3,20)$ is the difference the between L_R value of the compound and that of the ketalized compound, both as TMS derivatives; note the much lower value for the Δ14(11) and Δ411β compounds (*cf.* text).

A		B
5β-P-3α(11,20) D 598 (2776)	Reaction	5α-P-3β(11,20) D 756 (2878)
	DO	
95 5β-P-3α(11)DO(20) D 1132 (3053) $\Delta DO(20) = 277$ $R_b = 0.700$		**80** 5α-P-3β(11)DO(20) D 1446 (3160) $\Delta DO(20) = 282$ $R_b = 0.700$
	OX	
45 5β-P-3α(11)DO(20) Unreacted, removed by TLC	**45** 5β-P(3,11)DO(20) D,N 1093 (3029) $\Delta DO(20) = 286$ $R_b = 0.950$	On TLC fraction $R_b = 0.700$	**55** 5α-P(3,11)DO(20) D,N 1224 (3087) $\Delta DO(20) = 279$ $R_b = 0.950$	**25** 5α-P-3β(11)DO(20) Unreacted, removed by TLC
	WK	
6 5β-P-11β-DO(20) D,N 655 (2816) $R_b = 0.950$ Removed by TLC	**92** 5β-P(11)DO(20) D,N 572 (2757) $\Delta DO(20) = 277$ $R_b = 1.00$	On TLC fraction $R_b = 0.950$	**70** 5α-P(11)DO(20) D,N 634 (2802) $\Delta DO(20) = 278$ $R_b = 1.00$	**6** 5α-P-11β-DO(20) D,N 730 (2863) $R_b = 0.950$ Removed by TLC
	HY	
90 5β-P(11,20) D,N 302 (2480)		On TLC fraction $R_b = 1.00$	**82** 5α-P(11,20) D,N 334 (2524)	

Diagram 9. Synthesis of (A) 5β(11,20) and (B) 5αP(11,20). The percentages of the main products in the reaction mixtures are indicated; $\Delta DO(3,20)$ and $\Delta DO(20)$ are the differences between the L_R values of the compound and those of the ketalized compound (*cf.* text).

A		B
5β-P(11)DO(20)* D,N 572 (2757) $R_b = 1.00$ *Cf.* Diagram 9A	Reaction	5α-P(11)DO(20)* D,N 634 (2802) $R_b = 1.00$ *Cf.* Diagram 9B
	RD	
92 5β-P-11β-DO(20) D,N 655 (2816) $\Delta DO(20) = 250$ $R_b = 0.950$	(2 h)	**80** 5α-P-11β-DO(20) D,N 730 (2863) $\Delta DO(20) = 248$ $R_b = 0.950$
	HY	
90 5β-P-11β(20) D,N 368 (2506)	On TLC fraction $R_b = 0.950$	**80** 5α-P-11β(20) D,N 412 (2615)

Diagram 10. Synthesis of (A) 5β-P-11β(20) and (B) 5α-P-11β(20). The percentages of the main products in the reaction mixtures are indicated; $\Delta DO(20)$ is the difference between the L_R value of the compound and that of the ketalized compound. The asterisk (*) indicates a TLC fraction of R_b value 1.00 obtained with the reaction mixture of the WK step in Diagram 9.

A 5βP3α(11,20) D 598		Reaction	B 5αP3β(11,20) D 756	
		WK		
50 5βP3α11β D 419	**50** 5βP3α(11) D 365		**39** 5αP3β(11) D 460	**59** 5αP3β11β D 540
		OX		
25 5βP3α(11) D 365	**75** 5βP(3,11) D 344 L_R = 2536		**80** 5αP(3,11) D 603 L_R = 2780	**20** 5αP3β(11) D 460
		DO		
30 5βP3α(11) D 365 R_b = 0.788 Removed by TLC	**70** 5βP(11)DO(3) D 547 L_R = 2738 $\Delta DO(3)$ = 202 R_b = 1.00		**70** 5αP(11)DO(3) D 603 L_R = 2780 $\Delta DO(3)$ = 188 R_b = 1.00	**30** 5αP3β(11) D 460 R_b = 0.688 Removed by TLC
		RD		
	85 5βP11βDO(3) D 640 L_R = 2806 $\Delta DO(3)$ = 185	(2 h) on TLC fraction R_b = 1.00	**75** 5αP11βDO(3) D 712 L_R = 2852 $\Delta DO(3)$ = 173	
		HY		
	85 5βP11β(3) D 418 L_R = 2621		**75** 5αP11β(3) D 478 L_R = 2679	

Diagram 11. Synthesis of (A) 5βP11β(3) and (B) 5αP11β(3). The percentages of the concentrations of the main products only are given. $\Delta DO(3)$ is the difference between the L_R value of the compound and that of the ketalized compound.

WK. In general terms, effects of this reaction on (3), (11), and (20) were similar to those observed with (3), (11), and (17) in the androstane series[2]. The removal of (3) and (20) was essentially complete. Only a small proportion of these groups was reduced to OH through the side reaction (*cf.* Diagrams 1, 4, and 5, for example), and this reduction always followed a course similar to that of the RD reduction. On the other hand, (11) was not removed but underwent partial reduction to 11β, often to a large extent (*cf.* Diagrams 4–6, 9, and 11). When the desired WK product was that featuring (11), either this product was isolated through TLC (Diagram 9) or the 11β compound was reconverted to the 11-ketone by oxidation (Diagram 11).

DO. Ketalization of (3) and (20) was essentially complete under conditions described under Experimental; yields were much lower when previously described conditions[2] were used. As in the androstane series[2], (11) reacted very poorly if not at all. In the presence of (11), 20-ketones gave rise to a proportion of abnormal, as yet unidentified ketal, of which as much as 20% was observed in the case of 5αP3β(11,20) (*cf.* Diagram 9B). Upon hydrolysis of DO reaction mixtures obtained with (11,20) diketones, a new ketone appeared along with the initial one. Both the new ketone and

the corresponding abnormal ketal, and all products of the abnormal ketal appeared in the same proportion to the normal products in successive reactions, and appeared to undergo parallel modifications. While normal and corresponding abnormal products were not separable by TLC, the retention times and proportions of normal products were much higher in all cases.

ΔDO values were remarkably consistent. The average of *ΔDO*(20) in the presence of (11) was 280 ± 6 L_R units (Diagrams 7 and 9) and only 249 ± 8 in the presence of 11*β* (Diagrams 7 and 10). The normal value of *ΔDO*(3,20) in the presence of (11) was 476 ± 6 (Diagrams 8A and B), and 437 ± 3 in the presence of 11*β*. The much lower *ΔDO*(3,20) values with Δ4P(3,11,20) (Diagram 8C) indicated abnormal products. While the formation of Δ4PDO(3) from Δ4P(3) steroids has been demonstrated[5], hydrolysis of such ketals under present conditions[2] has exclusively yielded Δ4P(3) compounds in all cases. These were identified by their known retention times and that of their RD reduction products, *i.e.*, the corresponding Δ4P3*β* compounds. The latter were clearly distinguishable from the known retention times of corresponding Δ5P3*β* compounds.

From Diagram 11, the average *ΔDO*(3) values in the presence of (11) and 11*β* were 195 ± 7 and 180 ± 5 L_R units, respectively. Calculated as the difference between average *ΔDO*(3,20) and *ΔDO*(20) values, these *ΔDO*(3) values were 196 and 188, respectively, thus fairly close to those obtained directly.

The additivity, predictability, and structural specificity of *ΔDO* values confirm prior suggestions[2] as to their usefulness in steroid identification.

OX. As in the androstane series[2], CrO_3 oxidation under present conditions[2] of 11*β* to (11) was very rapid (Diagrams 4 and 5) and that of 20*α* and 20*β* to (20) somewhat less so (Diagram 6). With 3*α* or 3*β*, the reaction was never complete in 5 h (Diagrams 6, 9 and 11).

TMS. Hydroxyl groups 3*α*, 3*β*, 20*α* and 20*β* were completely derivatized under present conditions[2]. As in the androstane series[2], the retention time shift upon TMS derivatization was always positive, and 11*β* never formed a derivative (*cf.* N and D data in the diagrams). The advantages of TMS derivatization have been discussed[2].

HY. Hydrolyses performed under present conditions[2] were complete in all cases.

GLC data analysis and L_R values computations

Application of the methods of "*GLC data analysis*"[2] has established the patterns of G_R-normal and G_R-odd steroids clearly shown in Table XVIII. With a single exception, the steroids of groups P(20), P20*β*, and P20*α* are G_R-normal. Hence their L_R values can be accurately predicted from that of a single standard steroid in each group. As shown previously[2] the method consists in obtaining the G_R value of the group by subtracting M_R, taken from Table IV, from the L_R value of the standard. When, for instance, the 5*α*P3*β* members of the groups were chosen as standards, the G_R values thus obtained were those shown in brackets in Table XVI for the aforementioned groups. The L_R value of each steroid in these groups was then obtained as $L_R = M_R + G_R$ (eqn. 8 in ref. 2) where M_R is the appropriate value taken from Table IV. When compared to observed L_R values (Tables XIII, XIV, XV) the computed values showed errors which, expressed in seconds of retention time, have been listed in the last three columns of Table XVII.

TABLE IV

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

Steroid			M_R	*Source*
M	*Formula*	t'_{NR}		
I	5βP	129.5	2113	P5700
II	5αP	141	2150	P1800
III	5βP3β	252.5	2402	PREP; WK-5βP3β(20)
IV	5αP3α	252	2401	COMP; M_R 5αA3α** + 226*** = 2401
V	5βP(3)	258	2412	COMP; M_R 5βA(3)** + 226*** = 2412
VI	5βP3α	264	2421	P7800
VII	5αP(3)	284	2453	P4200
VIII	Δ4P3β	304	2483	COMP; M_R Δ4A3β** + 226*** = 2483
IX	Δ5P3β	314	2497	Q5350
X	5αP3β	321	2506	P3450
XI	Δ4P(3)	340	2531	COMP; M_R Δ4A(3)** + 226*** = 2531

* *Cf.* ref. 2, Table II.
** For M_R values, *cf.* ref. 2, Table I.
*** *Cf.* ref. 2, eqn. 17.

The (11)-featuring steroids showed G_R-odd patterns corresponding to the same M-features (Table XVIII), except for 5βP20β(11), which should have been G_R-normal. The pattern of G_R-odd steroids for 11β-featuring groups was identical also but corresponded to a different set of M-features. In the two sets of M-features, only V, VI, and VIII were common.

TABLE V

VALUES OF L_R AND G_R AND SOURCES OF STEROIDS OF GROUP P(11)

Steroid			L_R	G_R*	*Source*
M	*Formula*	t'_{NR}			
I	5βP(11)	184.5	2266	153	PREP; *cf.* Diagram 4
II	5αP(11)	201	2303	153	PREP; *cf.* Diagram 5
III	5βP3β(11)	366	2563	161	PREP; *cf.* Diagram 6
IV	5αP3α(11)	362	2559	159	COMP; L_R 5αA3α(11)*** + ΔG_R§
V	5βP(3,11)	344	2536	124**	PREP; *cf.* Diagram 6 and Table III
VI	5βP3α(11)	365	2562	141**	PREP; *cf.* Table III
VII	5αP(3,11)	391	2592	139**	PREP; *cf.* Table III
VIII	Δ4P3β(11)	420	2623	140**	COMP; L_R Δ4P3β20β(11)§§ − ΔG_R§
IX	Δ5P3β(11)	448	2651	154	COMP; M_R Δ5P3β§§§ + G_R P(11)*
X	5αP3β(11)	460	2663	157	PREP; *cf.* Table III
XI	Δ4P(3,11)	454	2655	123**	COMP; L_R Δ4P(3,11,20)§§ − ΔG_R†

* Average G_R-normal = G_R P(11) = 156.
** G_R-odd steroid.
*** Ref. 2, Table III.
§ ΔG_R = 232, *cf.* Table XVI.
§§ For L_R value, *cf.* appropriate table.
§§§ *Cf.* Table IV.
† For ΔG_R value, *cf.* Table XVI, last column.

TABLE VI

VALUES OF L_R AND G_R AND SOURCES OF STEROIDS OF GROUP P11β

Steroid			L_R	G_R*	*Source*
M	*Formula*	t'_{NR}			
I	5βP11β	211	2324	211**	PREP; *cf.* Diagram 4
II	5αP11β	234	2369	219	PREP; *cf.* Diagram 5
III	5βP3β11β	414	2617	215**	PREP; *cf.* Diagram 6 and Table III
IV	5αP3α11β	409	2612	211**	COMP; L_R 5αA3α11β*** + ΔG_R§
V	5βP11β(3)	418	2621	209**	PREP; *cf.* Diagram 11
VI	5βP3α11β	419	2622	201**	PREP; *cf.* Table III
VII	5αP11β(3)	478	2679	226	PREP; *cf.* Diagram 11
VIII	Δ4P3β11β	493	2692	209**	COMP; L_R Δ4P3β11β20β§§ − ΔG_R†
IX	Δ5P3β11β	522	2718	221	COMP; M_R Δ5P3β§§§ + G_R P11β*
X	5αP3β11β	540	2732	226	PREP; *cf.* Table III
XI	Δ4P11β(3)	563	2751	221	COMP; L_R ΔP11β(3,20)§§ − ΔG_R†

* Average G_R-normal = G_R P11β = 222.
** G_R-odd steroid.
*** Ref. 2, Table IV.
§ ΔG_R = 226, *cf.* Table XVI.
§§ For L_R value, *cf.* appropriate table.
§§§ *Cf.* Table IV.
† For ΔG_R value, *cf.* Table XVI.

TABLE VII

VALUES OF L_R AND G_R AND SOURCES OF STEROIDS OF GROUP P(11,20)

Steroid			L_R	G_R*	*Source*
M	*Formula*	t'_{NR}			
I	5βP(11,20)	303	2481	368	PREP; *cf.* Diagram 9A
II	5αP(11,20)	334	2524	374	PREP; *cf.* Diagram 9B
III	5βP3β(11,20)	598	2777	375	P7900
IV	5αP3α(11,20)	587	2769	368	COMP; L_R 5αA3α(11,17)*** + ΔG_R§
V	5βP(3,11,20)	572	2758	346**	Q500
VI	5βP3α(11,20)	598	2776	355**	P7850
VII	5αP(3,11,20)	650	2813	360**	P5630
VIII	Δ4P3β(11,20)	687	2837	354**	COMP; L_R Δ4P3β20β(11)§§ − ΔG_R§§§
IX	Δ5P3β(11,20)	728	2862	365	COMP; M_R Δ5P3β† + G_R P(11,20)*
X	5αP3β(11,20)	756	2878	372	P3600
XI	Δ4P(3,11,20)	740	2869	338**	Q4160

* Average G_R-normal = G_R P(11,20) = 370.
** G_R-odd steroid.
*** *Cf.* ref. 2, Table V.
§ ΔG_R = 235; *cf.* Table XVI.
§§ For L_R value, *cf.* Table IX.
§§§ For ΔG_R value, *cf.* Table XVI.
† *Cf.* Table IV.

TABLE VIII

VALUES OF L_R AND G_R AND SOURCES OF STEROIDS OF GROUP P11β(20)

Steroid			L_R	G_R*	*Source*
M	*Formula*	t'_{NR}			
I	5βP11β(20)	368	2566	453**	PREP; *cf.* Diagram 10A
II	5αP11β(20)	412	2615	466	PREP; *cf.* Diagram 10B
III	5βP3β11β(20)	725	2860	458**	PREP; *cf.* Diagram 7A
IV	5αP3α11β(20)	704	2847	446**	COMP; L_R 5αA3α11β(17)*** + ΔG_R§
V	5βP11β(3,20)	728	2862	450**	PREP; *cf.* Diagram 8A
VI	5βP3α11β(20)	723	2859	438**	PREP; *cf.* Diagram 7B
VII	5αP11β(3,20)	832	2920	465	PREP; *cf.* Diagram 8B
VIII	Δ4P3β11β(20)	858	2933	450**	COMP; L_R Δ4P3β11β20β§§ − ΔG_R§§§
IX	Δ5P3β11β(20)	910	2959	462	COMP; M_R Δ5P3β† + P11β(20)*
X	5αP3β11β(20)	938	2972	466	SRC; also PREP; *cf.* Diagram 7C
XI	Δ4P11β(3,20)	984	2993	462	Q3270; also PREP; *cf.* Diagram 8C

* Average G_R-normal = G_R P11β(20) = 464.
** G_R-odd steroid.
*** For L_R value, *cf.* ref. 2, Table VI.
§ ΔG_R = 213, *cf.* Table XVI.
§§ For L_R value, *cf.* Table XI.
§§§ For ΔG_R value, *cf.* Table XVI.
† For M_R value, *cf.* Table IV.

L/L plots[2] (Fig. 1a) clearly showed the relatedness of (11)-featuring groups and also the relatedness of 11β-featuring groups (Fig. 1b). As expected 5βP20β(11) (see above) did not obey the rule, nor did 5βP3α11β20β, which in this regard behaved as its 5βA3α11β17β counterpart by showing excess oddity[2]. It follows from these

TABLE IX

VALUES OF L_R AND G_R AND SOURCES OF STEROIDS OF GROUP P20β(11)

Steroid			L_R	G_R*	*Source*
M	*Formula*	t'_{NR}			
I	5βP20β(11)	408	2610	497**	PREP; *cf.* Table II
II	5αP20β(11)	457	2660	510	PREP; *cf.* Table II
III	5βP3β20β(11)	816	2912	510	PREP; *cf.* Diagram 3
IV	5αP3α20β(11)	820	2913	511	COMP; L_R 5αA3α11β(17)*** + ΔG_R§
V	5βP20β(3,11)	794	2900	488**	COMP; L_R 5βP(3,11,20)§§ + ΔG_R§§§
VI	5βP3α20β(11)	805	2906	485**	PREP; *cf.* Table II
VII	5αA20β(3,11)	904	2956	503**	COMP; L_R 5αP(3,11,20)§§ + ΔG_R§§§
VIII	Δ4P3β20β(11)	956	2980	503**	PREP; *cf.* Table II
IX	Δ5P3β20β(11)	1015	3007	510	COMP; M_R Δ5P3β† + G_R P20β(11)*
X	5αP3β20β(11)	1048	3020	514	PREP; *cf.* Table II
XI	Δ4P20β(3,11)	1028	3012	483**	COMP; L_R Δ4P(3,11,20)§§ + ΔG_R§§§

* Average G_R-normal = G_R P20β(11) = 511.
** G_R-odd steroid.
*** For L_R value, *cf.* ref. 2, Table VII.
§ For ΔG_R value, *cf.* Table XVI.
§§ For L_R value, *cf.* Table VII.
§§§ For ΔG_R value, *cf.* Table XVI.
† For M_R value, *cf.* Table IV.

TABLE X

VALUES OF L_R AND G_R AND SOURCES OF STEROIDS OF GROUP P20α(11)

Steroid			L_R	G_R*	*Source*
M	*Formula*	t'_{NR}			
I	5βP20α(11)	426	2629	516**	PREP; *cf.* Table II
II	5αP20α(11)	468	2670	520	PREP; *cf.* Table II
III	5βP3β20α(11)	850	2929	527	PREP; *cf.* Table II
IV	5αP3α20α(11)	844	2926	525	COMP; L_R 5αP3α20β(11)*** + 13§
V	5βP20α(3,11)	828	2918	514**	COMP; L_R 5βP20β(3,11)*** + 18§
VI	5βP3α20α(11)	845	2927	506**	PREP: *cf.* Table II
VII	5αP20α(3,11)	927	2967	514**	COMP; L_R 5αP20β(3,11)*** + 11§
VIII	Δ4P3β20α(11)	973	2988	505**	PREP; *cf.* Table II
IX	Δ5P3β20α(11)	1036	3015	518	COMP; L_R Δ5P3β20β(11)*** + 8§
X	5αP3β20α(11)	1068	3029	523	PREP; *cf.* Table II
XI	Δ4P20α(3,11)	1052	3022	491**	COMP; L_R Δ4P20β(3,11)*** + 10§

* Average G_R-normal = G_R P20α(11) = 523.
** G_R-odd steroid.
*** For L_R value, *cf.* Table IX.
§ *Cf.* Table XVIII and text.

relationships that L_R values of (11)-featuring steroids on the one hand, and 11β-featuring steroids on the other hand were accurately calculated through the ΔG_R method[2] from the L_R values of a few selected available standards by procedures previously used with the androstane (17)- and 17β-counterparts. The accuracy of these computations using the ΔG_R values listed in Table XVI were clearly predictable from the precision of L/L plots (Figs. 1a and 1b).

TABLE XI

VALUES OF L_R AND G_R AND SOURCES OF STEROIDS OF GROUP P11β20β

Steroid			L_R	G_R*	*Source*
M	*Formula*	t'_{NR}			
I	5βP11β20β	442	2645	532**	PREP; *cf.* Table I
II	5αP11β20β	500	2699	549	PREP; *cf.* Table I
III	5βP3β11β20β	874	2941	540**	PREP; *cf.* Diagram 2 and Table I
IV	5αP3α11β20β	874	2942	540**	COMP; L_R 5αA3α11β17β*** + ΔG_R§
V	5βP11β20β(3)	891	2950	536**	COMP; L_R 5βP11β(3)§§ + ΔG_R§§§
VI	5βP3α11β20β	868	2938	517**	PREP; *cf.* Table I
VII	5αP11β20β(3)	1024	3010	557	COMP; L_R 5αP11β(3)§§ + ΔG_R§§§
VIII	ΔP3β11β20β	1060	3025	542**	PREP; *cf.* Table I
IX	Δ5P3β11β20β	1123	3050	553	COMP; M_R Δ5P3β† + G_R P11β20β*
X	5αP3β11β20β	1158	3064	558	PREP; *cf.* Table I
XI	Δ4P11β20β(3)	1210	3084	552	COMP; L_R Δ4P11β(3,20)†† + ΔG_R§§§

* Average G_R-normal = G_R P11β20β = 555.
** G_R-odd steroid.
*** For L_R value, *cf.* ref. 2, Table VIII.
§ For ΔG_R value, *cf.* Table XVI.
§§ For L_R value, *cf.* Table VI.
§§§ For ΔG_R value, *cf.* Table XVI.
† For M_R value, *cf.* Table IV.
†† For L_R value, *cf.* Table VIII.

TABLE XII

VALUES OF L_R AND G_R AND SOURCES OF STEROIDS OF GROUP P11β20α

Steroid			L_R	G_R*	*Source*
M	*Formula*	t'_{NR}			
I	5βP11β20α	499	2698	585**	PREP; *cf.* Table I
II	5αP11β20α	557	2745	595	PREP; *cf.* Table I
III	5βP3β11β20α	981	2992	590**	PREP; *cf.* Diagram 2
IV	5αP3α11β20α	978	2991	591**	COMP; L_R 5αP3α11β20β*** + 49§
V	5βP11β20α(3)	1010	3004	592**	COMP; L_R 5βP11β20β(3)*** + 54§
VI	5βP3α11β20α	985	2993	572**	PREP; *cf.* Table I
VII	5αP11β20α(3)	1140	3057	604	COMP; L_R 5αP11β20β(3)*** + 47§
VIII	Δ4P3β11β20α	1171	3068	585**	PREP; *cf.* Table I
IX	Δ5P3β11β20α	1243	3094	597	COMP; L_R Δ5P3β11β20β*** + 44§
X	5αP3β11β20α	1278	3107	600	PREP; *cf.* Table I
XI	Δ4P11β20α(3)	1350	3130	599	COMP; L_R Δ4P11β20β(3)*** + 46§

* Average G_R-normal = G_R P11β20α = 599.
** G_R-odd steroid.
*** For L_R value, *cf.* Table XI.
§ See Table XVIII and text.

The important new fact arising from the present data was the relatedness of steroids of the androstane series previously described[2] with their pregnane counterparts in the present article, according to the following pattern:

A(11)/P(11)	and	A11β/P11β
A(11,17)/P(11,20)		A11β(17)/P11β(20)
A17β(11)/P20β(11)		A11β17β/P11β20β

TABLE XIII

VALUES OF L_R AND G_R AND SOURCES OF STEROIDS OF GROUP P(20)

Steroid			L_R	G_R*	*Source*
M	*Formula*	t'_{NR}			
I	5βP(20)	221	2344	231	SRC
II	5αP(20)	242	2384	234	SRC
III	5βP3β(20)	435	2638	236	P8180
IV	5αP3α(20)	434	2637	235	COMP; M_R 5αP3α** + 234*
V	5βP(3,20)	439	2643	231	P7150
VI	5βP3α(20)	455	2658	237	P8150
VII	5αP(3,20)	495	2690	237	P2750
VIII	Δ4P3β(20)	521	2717	234	COMP; M_R Δ4P3β** + 234*
IX	Δ5P3β(20)	535	2728	231	Q5500
X	5αP3β(20)	554	2743	233	P3830
XI	Δ4P(3,20)	582	2765	233	Q2600

* Average G_R-normal = G_R P(20) = 234.
** For M_R value, *cf.* Table IV.

TABLE XIV

VALUES OF L_R AND G_R AND SOURCES OF STEROIDS OF GROUP P20β

Steroid			L_R	G_R*	*Source*
M	*Formula*	t'_{NR}			
I	5βP20β	290	2463	350	PREP; RD (2 h)*** 5βP(20)§
II	5αP20β	317	2502	352	SRC
III	5βP3β20β	566	2753	353	P6140
IV	5αP3α20β	573	2758	354	P2000
V	5βP20β(3)	572	2757	355	SRC
VI	5βP3α20β	585	2767	346**	P6050
VII	5αP20β(3)	642	2807	354	P4000
VIII	Δ4P3β20β	686	2836	353	Q1490
IX	Δ5P3β20β	709	2851	354	Q4490
X	5αP3β20β	728	2862	356	P2100
XI	Δ4P20β(3)	773	2888	357	Q3630

* Average G_R-normal = G_R P20β = 353.5.
** G_R-odd steroid.
*** *Cf.* Table I.
§ *Cf.* Table XIII.

This relatedness, clearly indicated by L/L plots (Figs. 2c and d) described by the relationship

$$L_R(\mathrm{P}) = L_R(\mathrm{A}) + \Delta G_R$$

was expressed by corresponding ΔG_R values obtained as differences of L_R values, as the above expression indicates, for M-corresponding steroids in each related group pair. For the present demonstration, the 5α3β-steroids were used as standards for ΔG_R calculation (*cf.* Table XVII); the resulting ΔG_R values, listed in Table XVI,

TABLE XV

VALUES OF L_R AND G_R AND SOURCES OF STEROIDS OF GROUP P20α

Steroid			L_R	G_R*	*Source*
M	*Formula*	t'_{NR}			
I	5βP20α	311	2493	380	PREP; RD (2 h)** 5βP(20)***
II	5αP20α	338	2529	379	SRC
III	5βP3β20α	606	2782	380	P6100
IV	5αP3α20α	611	2786	384	P1950
V	5βP20α(3)	617	2790	378	SRC
VI	5βP3α20α	634	2802	381	P6000
VII	5αP20α(3)	681	2833	380	SRC
VIII	Δ4P3β20α	725	2860	377	PREP; RD (2 h)** Δ4P(3,20)***
IX	Δ5P3β20α	748	2874	377	Q4460
X	5αP3β20α	770	2886	380	P2050
XI	Δ4P20α(3)	820	2913	382	Q3600

* Average G_R-normal = G_R P20β = 380.
** *Cf.* Table I.
*** *Cf.* Table XIII.

TABLE XVI

G_R AND ΔG_R VALUES

Group	G_R*	ΣG_R**	Group pair	ΔG_R***	Group	G_R§	ΔG_R§§	
							$G_R P-G_R A+226$	$L_R P-L_R A$
P(11)	156		P(11,20)/P(11)	214	A(11)	150.5	231.5	232
P11β	222		P20β(11)/P(11)	357	A11β	222	226	228
P(11,20)	370	390	P20β(11)/P(11,20)	143	A(11,17)	361	235	235
P11β(20)	464	456	P11β(20)/P11β	242	A11β(17)	477	213	213
P20β(11)	511	509.5	P11β20β/P11β	333	A17β(11)	507	230	235
P11β20β	555	575.5	P11β20β/P11β(20)	91	A11β17β	568	213	214
P11β20α	599	602						
P(20)	234							
	(233)							
P20β	353.5							
	(356)							
P20α	380							
	(380)							

* G_R values of the groups listed in column 1 (*cf.* footnote * in Tables V–XV); values in brackets for P(20), P20β, and P20α are the G_R values of the 5αP3β-compounds in each group.

** The ΣG_R value is the sum of the G_R values corresponding to the functional groups of the component (ref. 1); example: ΣG_R P(11,20) = G_R P(11) + G_R P(20).

*** The ΔG_R values in this column are the differences in G_R value for groups of the pair.

§ G_R values of the groups in column 6 taken from footnote * in Table III–VIII, ref. 2.

§§ ΔG_R values for corresponding groups in columns 1 and 6 computed from G_R values (column 8) and from L_R values (column 9) of 5αP3β members of the groups (see text).

TABLE XVII

ERRORS IN SECONDS OF RETENTION TIME OBSERVED IN L_R VALUES COMPUTED FROM GLC DATA OBTAINED WITH STANDARD STEROIDS S*

Steroid		(11)-Featuring groups			11β-Featuring groups			Position 20		
M	Formula	(11)	(11,20)	20β(11)	11β	11β(20)	11β20β	(20)	20β	20α
I	5βP	−0.4	+0.8	+0.5**	+0.3**	−0.5**	+0.6**	+0.6	+1.4	0
II	5αP	+1.2	−0.8	+3	+1.2	−0.6	+1.3	−0.3	+1.2	−0.4
III	5βP3β				**	**	**	−1.6	+1.3	0
IV	5αP3α				**	**	**	−1.2	0	−3.5
V	5βP(3)	+2**	+1.8**	+3.6**	+2.0**	−3**	−1.3**	+1.2	−0.8	+1.7
VI	5βP3α	−2**	0**	0**	+1.1**	+1.0**	S**	−2.4	S**	−0.8
VII	5αP(3)	0**	0**	0**	+1.3	−1.2	−1.3	−2.4	0	0
VIII	Δ4P3β	−0.6**	−3**	+1.3**	+0.7**	+1.3**	0**	0	+1.0	+3
IX	Δ5P3β	−0.6	−5	0	0	0	−1.3	+2	S	+3
X	5αP3β	S	S	S	S	S	S	S	−2.0	S
XI	Δ4P(3)	−1.2**	−5**	−1.3**	0	+1.3	0	0	−3.2	−2.4

* Differences observed between computed L_R values and L_R values listed in Tables V–XV were obtained in L_R units. One L_R unit corresponds to 0.233% of the retention time, t'_{NR}, or 0.14 × t'_{NR} seconds when t'_{NR} is expressed in minutes. No error is given for compounds used as standards (S) or for 5βP3β- and 5αP3α-compounds (see text).

** G_R-odd steroid.

TABLE XVIII

OBSERVED AND COMPUTED VALUES OF DIFFERENCES BETWEEN L_R VALUES OF M-CORRESPONDING 20α- AND 20β-STEROIDS

Steroid		L_R 20α − L_R 20β		L_R 20α(11) − L_R 20β(11)		L_R 11β20α − L_R 11β20β	
M	*Formula*	*Observed**	ε**	*Observed****	*Computed*§	*Observed*§§	*Computed*§
I	5βP	30	+2	19	15	53	51
II	5αP	27	−1	10	12	46	48
III	5βP3β	29	+1	17	14	51	50
IV	5αP3α	28	0		13		49
V	5βP(3)	33	+5		18		54
VI	5βP3α	35	+7	21	20	57	56
VII	5αP(3)	26	−2		11		47
VIII	Δ14P3β	24	−4	8	9	43	45
IX	Δ15P3β	23	−5		8		44
X	5αP3β	24	−4	7	9	43	45
XI	Δ14P(3)	25	−3		10		46
		Mean 28		Mean 13		Mean 49	

* From L_R values listed in Tables XIV and XV, respectively.
** Deviation from the mean.
*** From L_R values listed in Tables IX and X, respectively.
§ By adding ε to the mean.
§§ From L_R values listed in Tables XI and XII, respectively.

last column, then served to compute through the above expression the L_R values of the pregnane steroids, L_R(P), from L_R values of their M-corresponding androstane counterparts, L_R(A), taken from Tables III–VIII in ref. 2. A comparison with observed values listed in Tables V–XI has revealed errors. These, expressed in seconds of retention time, are listed in Table XVII. Of course, no error could be listed for the 5αP3β-compounds used as standards. Because the L_R values of 5βA3β and 5αP3α steroids given in relevant tables were computed, not observed values, obtained by the above method from observed 5βP3β- and 5αA3α-values, respectively, they could not be used for the purpose of evaluating errors arising from the application of this method. Examination of errors listed in Table XVII shows most of them to be no larger than 3 sec; neither of the two exceptionally large 5-sec errors exceeds 1% of the corresponding retention time.

The L_R values of P20α(11) and P11β20α groups can be adequately computed from the L_R values of corresponding groups featuring 20β. This is shown in Table XVIII, where differences of L_R values of corresponding 20α- and 20β-featuring steroids are listed. For groups P20α and P20β, for example, the difference in L_R values of M-corresponding steroids varies from a mean of 29 L_R units by a variable quantity ε. Remarkably, differences of L_R values for P20α(11) and P20β(11) steroids on the one hand, and for P11β20α and P11β20β steroids on the other hand, also vary from the corresponding mean by the same quantity ε. Most steroids in the P20β and P20α groups are commercially available. Hence ε can be obtained for all M-features listed in Table XVIII. The L_R values of unavailable steroids in the P20α(11) and P11β20α groups therefore were computed by adding to the L_R values of their 20β counterparts the mean difference + ε, *i.e.*, the computed differences listed in Table XVIII. As seen

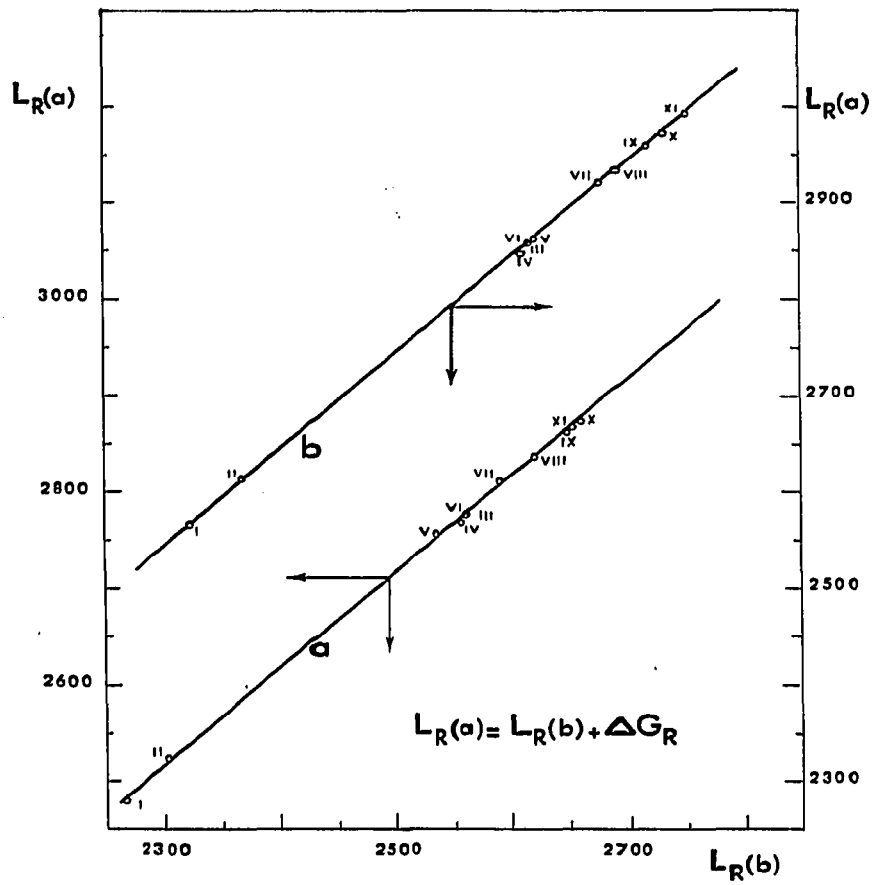

Fig. 1. Examples of L/L plot. Roman numerals indicate corresponding M-features. (a) L_R (a), group P11) (for L_R values, *cf.* Table V); L_R (b), group P(11,20) (Table VII). (b) L_R(a), group P11β (Table (VI); L_R(b), group P11β(20) (Table VIII).

from this table, computed and observed differences of available pairs agree within narrow limits.

The properties of G_R-odd steroids which emerge from the data thus far presented in this series can be summarized as follows. Oddity seldom arises when G-features do not include (11) or 11β. In contrast to (17), 17β, (20), 20β, and 20α, functional groups (11) and 11β induce extensive oddity. In all groups featuring (11), G_R-odd steroids correspond to specific M-features. Similarly, 11β induces a specific pattern of G_R-odd steroids which differs from that induced by (11). Expressed in L_R units, the extent of oddity, or departure of the G_R value from the normal one[2], varies in both patterns according to what M-feature is present. Yet, the extent of oddity is quantitatively constant for all M-corresponding steroids featuring (11), and similarly, for those which feature 11β. Since such steroids may include either (17), 17β, (20), 20β or 20α, it is evident that the extent of oddity is not affected by these functional groups, nor by modifications to the carbon skeleton, from androstane to pregnane, for example.

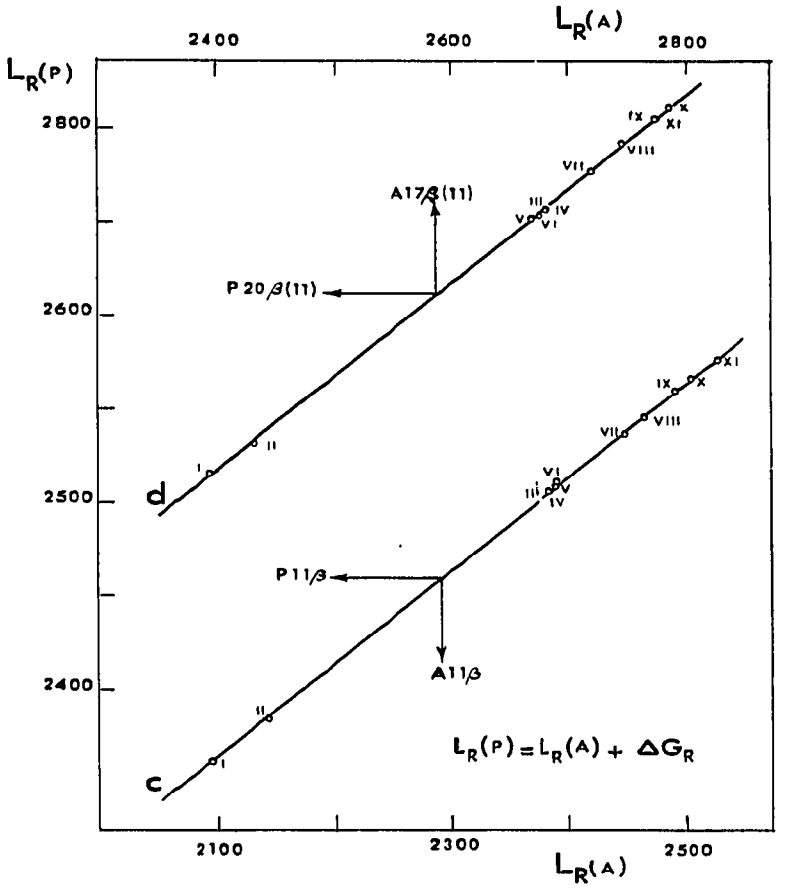

Fig. 2. Examples of L/L plot demonstrating relatedness of groups of the pregnane (P) and androstane (A) series. Roman numerals indicate corresponding M-features. (c) L_R(P), group P11β (Table VI; L_R(A), group A11β (Table IV, ref. 2). (d) L_R(P), group P20β(11) (Table IX); L_R(A), group A17β(11) (Table VII in ref. 2).

The constancy of oddity induced by (11) or 11β is the underlying reason for the occurrence of group relatedness characterized by ΔG_R values[2]. Hence, it is the key to the simple, accurate computations of L_R values which have been previously demonstrated. A general method for the identification of steroids of natural origin requires, however, that similar computations be applicable to all possible combinations of functional groups which occur in these compounds. Further articles of this series will show that the extent of oddity induced by other functional groups, for example 11α, 16α, and 16β, is constant also when these functional groups coexist with one or more functional groups which do not induce oddity, including 17α and 21-OH in the pregnane series.

In view of numerous possible combinations of the functional groups mentioned above, the aquisition and testing of a considerable amount of data is a prerequisite to an unequivocal demonstration of constancy of oddity, and consequently, to the publication of the general method of steroid identification described in this series.

In contrast to GLC data obtained by current methods, the numerous L_R values presented in this series are reproducible within $\pm$ 1 L_R unit in any laboratory where the present normal conditions are used[2]. For the purpose of steroid identification, such data and the derived G_R and ΔG_R values are equivalent to accurate physical constants.

The use of different normal conditions or column packing under previously defined specifications[2] leads to numerically different systems of L_R, G_R and ΔG_R values which serve equally well the purpose of steroid identification[2]. The present data should be helpful in establishing equivalent systems which in many respects are qualitatively similar to the present one.

The data on steroid preparation should be useful, if only for describing procedures to obtain samples of commercially unavailable steroids for the purpose of steroid identification. The course of simple classical reactions with functional groups studied in the present work will be reviewed at the conclusion of this series and their usefulness in the positive identification of structures suggested by retention times will be demonstrated.

Data acquisition systems including computer memory have become current features of GLC. Because all computations used in the present method are based on simple linear relations directly amenable to digitalization, operations such as the correction of retention times and the selection of probable structures corresponding to observed t'_{NR} values could be easily programmed with the present data stored in such systems.

GLC–high-resolution mass spectrometry is the current method of choice for steroid identification. The present method is undoubtedly simpler, faster, and less demanding in equipment, personnel, sample size and steroid resolution[2], and should prove very effective in any situation where speed and cost are serious considerations.

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REFERENCES

1 F. A. Vandenheuvel and A. S. Court, *J. Chromatogr.*, 38 (1968) 439; 39 (1969) 1.
2 F. A. Vandenheuvel, *J. Chromatogr.*, 96 (1974) 47.
3 F. A. Vandenheuvel, *J. Chromatogr.*, 38 (1968) 373.
4 F. A. Vandenheuvel, G. L. Hinderks and J. C. Nixon, *J. Amer. Oil Chem. Soc.*, 42 (1965) 283.
5 J. J. Brown, R. H. Lenhard and S. Bernstein, *J. Amer. Chem. Soc.*, 86 (1964) 2183.