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

IV. SUBSTITUTION IN THE PREGNANE SIDE-CHAIN*

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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 are described. Reactions studied include the conversion of keto groups to hydroxyl groups by $NaBH_4$, and to dioxolone derivatives by 1,2-diethanol; 17α-hydroxylation of C20-ketosteroids; the conversion of hydroxyl groups to trimethylsilyl (TMS) ethers by hexamethyldisilazane; the hydrolysis of dioxolone and TMS derivatives by H^+. Effects of the Wolff–Kishner reagents, and CrO_3 were also studied. GLC chromatograms of reaction mixtures of single- and multistep reactions readily provide information on effects on functional groups at positions 3, 17, 20, and 21 in the pregnane series, and the retention times of many steroids unavailable from commercial and 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 fourth of a series of communications[1–3] dealing with reactions and structurally dependent chromatographic properties of steroids, the present article concerns groups of steroids substituted in the pregnane side-chain. These compounds, which feature a 20-keto or a 20α- or 20β-hydroxyl group alone or in conjunction with a 17α- or 21-hydroxyl group, or both, include hormones and metabolites of considerable importance to studies of animal reproduction.

As the present data show, steroids of these groups are essentially G_R-normal[1,2], *i.e.*, their G_R value defined as

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

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TABLE I

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

Steroid			*Source*
M	*Abbreviation*	M_R	
I	5βP	2113	P5700
II	5αP	2150	P1800
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	P7800
VII	5αP(3)	2453	P4200
VIII	Δ4P3β	2483	Calculated; M_R Δ4A3β** + 226*** = 2483
IX	Δ5P3β	2497	Q5350
X	5αP3β	2506	P3450
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.

is a constant characteristic of the group. Hence their L_R value, defined by

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

and, consequently, their retention time t'_{NR} under standardized conditions[1] is readily obtained by

$$L_R = M_R + G_R \qquad \text{(eqn. 8 in ref. 1)}$$

with M_R taken from Table I.

One purpose of the present article is to show that for *M*-corresponding steroids[1] of two groups, a and b, the general relationship

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

holds. Hence, L_R(a), the L_R value of any steroid in one group, can be accurately calculated from L_R(b), the L_R value of the *M*-corresponding steroid in another group, and the ΔG_R(a,b) value of the group pair. Simple methods for obtaining the G_R and ΔG_R values given in Table XIV have been described[1].

Detailed definitions of other symbols and abbreviations used in the present article are found in ref. 1.

The gas–liquid chromatographic (GLC) properties of steroids of groups P(20), P20β, P20α, P17α(20), P17α20β, P17α20α, P21(20), P20β21, P20α21, P17α20β21 and P17α20α21, listed in Tables III–XIII, respectively, were obtained with trimethylsilyl ethers (TMS) of commercially available standards**, gifts from the Steroid Reference Collection***, or steroids synthesized in this laboratory.

Although most of the synthesized compounds were obtained through conversion of keto groups to hydroxyl groups by $NaBH_4$ (RD reaction) (Table II) and substitution of P(20)-steroidsby 17α-hydroxyl (Diagrams 1–3), effects of various other

* The L_R value shown here is defined in ref. 1 as the logarithmic expression of t'_{NR} and as such should include the log sign as shown above. Unfortunately, this sign was left out from eqns. 6 and 7 in ref. 1.

** In the tables, under Source, a letter followed by four digits indicates catalogue number of Steraloids Inc., P.O. Box 127, Pawling, N.Y. 12564, U.S.A.

*** Indicated by SRC under Source in the tables; see Acknowledgements.

TABLE II

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

Starting material			*Normal products**	
Abbreviation	*Source*	*GLC properties*	*20α/20β ratio*	*GLC properties*
βP17α(20)	*Cf.* Diagram 1, A	*Cf.* Table VI	36/64	*Cf.* Tables VII(β) and VIII(α)
αP17α(20)	*Cf.* Diagram 1, B	(P17α(20) group)	36/64	
3P3β17α(20)	P6810; *cf.* Diagram 2, A		36/64	
βP17α(3,20)	P8090		40/60	
βP3α17α(20)	P6570; *cf.* Diagram 2, B		36/64	
5P3β17α(20)	*Cf.* Diagram 3, B		38/62	
αP3β17α(20)	P2490; *cf.* Diagram 3, A		36/64	
14P17α(3,20)	Q3360		40/60	
5βP21(3,20)	P8120	*Cf.* Table IX	*Cf.* Text	*Cf.* Tables X(β) and XI(α)
5βP3α21(20)	P6920, SRC	(P21(20) group)	13/87	
5αP21(3,20)	P3750		15/85	
15P3β21(20)	P4780, SRC		15/85	
14P21(3,20)	Q3460		17/83	
5βP17α21(20)	SRC	Decomposes	24/76	*Cf.* Tables XII(β) and XIII(α)
5βP17α21(3,20)	P6300	(P17α21(20) group)	*Cf.* text	
5αP17α21(3,20)	P2320		23/77	
14P17α21(3,20)	Q1610		19/81	

* While the RD reduction of (20) invariably produces both 20α and 20β isomers, that of (3) yields almost exclusively 3α with a 5β compound and 3β with a 5α compound. Minor products are discussed in the text.

reactions previously described[1,2] have been studied at the submicrogram level. The results are discussed with regard to possible uses of these reactions in steroid identification.

EXPERIMENTAL

Reactions

Procedures used for the reduction of keto groups by sodium borohydride (RD), their reductive removal by the Wolff–Kishner reaction (WK), their formation from hydroxyl groups by oxidation using chromium trioxide (OX), the TMS derivatization of hydroxyl groups, and the hydrolysis of TMS and dioxolone derivatives have been described in detail[1].

The procedure described[1] for the preparation of dioxolone (DO) derivatives of ketones was modified: only 0.1 mg of *p*-toluenesulfonic acid (PTSA) was used, and the reaction time extended to 7 h, with hourly addition of toluene (see Discussion).

The procedure used for the 17α-substitution of P(20)-steroids included three steps (*cf.* Fig. 1).

Step 1 (Ac_2O/PTSA)

From 0 to 1 mg of steroid placed in a 1-ml tube was dissolved in 100 μl of a solution of *p*-toluenesulfonic acid in acetic anhydride (0.6 g PTSA in 50 ml Ac_2O). The tube was placed in the stainless-steel pressure vessel described in ref. 1 p. 77, together with a 5-ml vial containing 4 ml Ac_2O. The vessel was filled with nitrogen, closed (*cf.* ref. 1, p. 78), placed in an oven, heated to 142° for 4 h, and allowed to cool

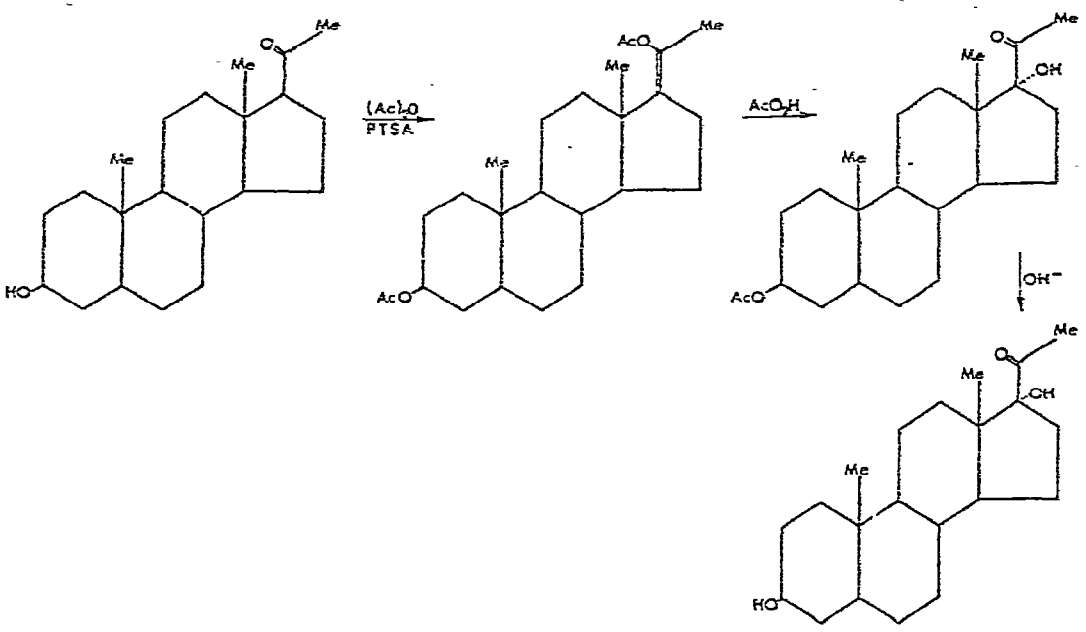

Fig. 1. Introduction of the 17α-hydroxyl group in 20-ketosteroids by the method of Kritchevsky and Gallagher[4] as modified by Oliveto and Hershberg[5].

to room temperature. The Ac_2O was removed from the reaction mixture under vacuum, and 350 μl of benzene were stirred with the residue. The solution was washed by stirring with 350 μl of water. Most of the solvent was evaporated under nitrogen (56°), and the residue dried over P_2O_5 under vacuum for 2 h.

Step 2 (AcO_2H)

One hundred microliters of a solution of peracetic acid in benzene (5 ml

TABLE III

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

Steroid					*Source*
M	*Abbreviation*	t'_{NR}	L_R	G_R	
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	Calculated; M_R 5αP3α** − 234*
V	5βP(3,20)	439	2643	231	P7150
VI	5βP3α(20)	455	2658	237	P8150
VII	5αP(3,20)	490	2690	237	P2750
VIII	Δ4P3β(20)	521	2717	234	Calculated; 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 I.

TABLE IV

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

Steroid					*Source*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP20β	290	2463	350	Prepared; 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	345**	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.* ref. 2, Table I.
§ *Cf.* Table III.

AcO_2H + 25 ml C_6H_6) were stirred with the enol acetate and the stoppered tube left at room temperature for 2.5 h. Thirty microliters of Na_2SO_3 solution (40 g Na_2SO_3 + 150 ml water) and 200 μl C_6H_6 were stirred with the reaction mixture. After adding 300 μl water, stirring and centrifuging, the benzene layer was removed and washed twice with 300 μl of water. The solvent was evaporated under nitrogen (56°).

Step 3 (OH^-)

Saponification of the residue was achieved by heating (56°) for 30 min with 100 μl of methanolic NaOH (1 g NaOH, 10 ml water, 90 ml methanol), evaporating

TABLE V

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

Steroid					*Source*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP20α	311	2493	380	Prepared; 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	Prepared; 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.* ref. 2, Table I.
*** *Cf.* Table III.

TABLE VI

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P17α(20)

Steroid					*Source*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP17α(20)	317	2501	388	Prepared; *cf.* Diagram 1, A
II	5αP17α(20)	350	2544	394	Prepared; *cf.* Diagram 1, B
III	5βP3β17α(20)	607	2783	381**	P6810; prepared: *cf.* Diagram 2, A
IV	5αP3α17α(20)	617	2790	389	Calculated; L_R 5αP3α(20)*** + ΔG_R‡
V	5βP17α(3,20)	629	2798	386	P8090
VI	5βP3α17α(20)	618	2791	370**	P6570; prepared; *cf.* Diagram 2, B
VII	5αP17α(3,20)	696	2842	389	Calculated; L_R 5αP(3,20)*** − ΔG_R‡
VIII	Δ4P3β17α(20)	746	2872	389	Calculated: L_R Δ4P3β(20)*** + ΔG_R‡
IX	Δ5P3β17α(20)	770	2886	389	Calculated; L_R Δ5P3β(20)*** + ΔG_R‡
X	5αP3β17α(20)	790	2897	391	P2490; prepared; *cf.* Diagram 3, A
XI	Δ4P17α(3,20)	859	2934	403	Q3360

* Average G_R-normal value = G_R P17α(20) = 389.
** G_R-odd steroid.
*** For L_R value, see Table III.
‡ For ΔG_R value, see Table XIV.

under nitrogen (56°), stirring the residue with 500 μl $CHCl_3$, removing the extract, evaporating most of the $CHCl_3$ under nitrogen (56°), and drying over P_2O_5 *in vacuo*.

Gas–liquid and thin-layer chromatography

Both gas–liquid and thin-layer chromatographic (TLC) methods were used as previously described[1,2]. All t'_{NR} values were obtained with steroids or steroid mixtures submitted to the TMS derivatization procedure.

TABLE VII

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P17α20β

Steroid					*Source*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP17α20β	438	2641	526	Prepared; *cf.* Table II
II	5αP17α20β	475	2677	527	Prepared; *cf.* Table II
III	5βP3β17α20β	837	2923	521**	Prepared; *cf.* Table II
IV	5αP3α17α20β	861	2935	534	P5000
V	5βP17α20β(3)	855	2932	520**	Calculated; L_R 5βP20β(3)*** + ΔG_R‡
VI	5βP3α17α20β	853	2931	510**	P9480; prepared; *cf.* Table II
VII	5αP17α20β(3)	960	2982	529	Calculated; L_R 5αP20β(3)*** + ΔG_R‡
VIII	Δ4P3β17α20β	1024	3010	527	Prepared; *cf.* Table II
IX	Δ5P3β17α20β	1057	3024	527	Q5890‡‡; prepared; *cf.* Table II
X	5αP3β17α20β	1089	3037	531	Prepared; *cf.* Table II
XI	Δ4P17α20β(3)	1154	3062	532	Q1850

* Average G_R-normal value = G_R P17α20β = 530.
** G_R-odd steroid.
*** For L_R value, see Table IV.
‡ For ΔG_R value, see Table XIV.
‡‡ Designated Δ5P3β17α20α in Steraloids catalogue: Δ5P3β17α20α obtained from SRC (*cf.* Table VIII) had the expected retention time.

TABLE VIII

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P17α20α

Steroid					*Source*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP17α20α	463	2666	552	Prepared; *cf.* Table II
II	5αP17α20α	503	2702	552	Prepared; *cf.* Table II
III	5βP3β17α20α	904	2956	554	Prepared; *cf.* Table II
IV	5αP3α17α20α	910	2959	552	P4950
V	5βP17α20α(3)	921	2964	552	Calculated; L_R 5βP20α(3)** + ΔG_R***
VI	5βP3α17α20α	939	2973	552	P9450; prepared; *cf.* Table II
VII	5αP17α20α(3)	1012	3005	552	Calculated; L_R 5αP20α(3) + ΔG_R***
VIII	Δ4P3β17α20α	1079	3033	550	Prepared; *cf.* Table II
IX	Δ5P3β17α20α	1116	3048	551	SRC: prepared: *cf.* Table II
X	5αP3β17α20α	1150	3060	554	Prepared; *cf.* Table II
XI	Δ4P17α20α(3)	1226	3088	557	Q1820

* Average G_R-normal value = G_R P17α20α = 554.
** For L_R value, see Table V.
*** For ΔG_R value, see Table XIV.

DISCUSSION

Reactions

RD. Reduction by $NaBH_4$ always produced both the 20β- and 20α-isomers as major products (Table II) in proportions characteristic of the group involved. Assignments for GLC peaks of reduction products could be made readily since a number of standard 20α- and/or 20β-isomers were available in all groups (*cf.* Tables III–XIII). The 20α/20β ratio of the products was highest (average: 37/63) with P17α(20)-steroids as starting materials, lowest (average: 15/85) with P21(20)- and

TABLE IX

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P21(20)

Steroid					*Source*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP21(20)	498	2698	585	Calculated: L_R 5βP(20)*** + ΔG_R§
II	5αP21(20)	547	2738	588	Calculated; L_R 5αP(20)*** + ΔG_R§
III	5βP3β21(20)	982	2992	590	Calculated; L_R 5βP3β(20)*** + ΔG_R§
IV	5αP3α21(20)	980	2991	590	Calculated; L_R 5αP3α(20)*** + ΔG_R§
V	5βP21(3,20)	958	2981	569**	P8120
VI	5βP3α21(20)	953	2979	558**	P6920 and SRC
VII	5αP21(3,20)	1103	3042	589	P3750
VIII	Δ4P3β21(20)	1178	3071	588	Calculated; L_R Δ4P3β(20)*** + ΔG_R§
IX	Δ5P3β21(20)	1221	3086	589	P4780 and SRC
X	5αP3β21(20)	1247	3096	590	Calculated; L_R 5αP3β(20)*** + ΔG_R§
XI	Δ4P21(3,20)	—	Decomposes		Q3460

* Average G_R-normal value = G_R P21(20) = 589.
** G_R-odd steroid.
*** For L_R value, see Table III.
§ For ΔG_R value, see Table XIV.

TABLE X

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P20β21

Steroid					*Source*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP20β21	617	2790	677	Calculated; L_R 5βP20β*** + ΔG_R§
II	5αP20β21	672	2827	677	SRC
III	5βP3β20β21	1203	3080	678	Calculated; L_R 5βP3β20β*** + ΔG_R§
IV	5αP3α20β21	1217	3085	684	Calculated; L_R 5αP3α20β*** + ΔG_R§
V	5βP20β21(3)	1214	3084	672**	Calculated; L_R 5βP20β(3)*** + ΔG_R§
VI	5βP3α20β21	1203	3080	659**	Prepared: *cf.* Table II
VII	5αP20β21(3)	1361	3134	681	Calculated; L_R 5αP20β(3)*** + ΔG_R§
VIII	Δ4P3β20β21	1460	3164	682	Prepared: *cf.* Table II
IX	Δ5P3β20β21	1507	3178	681	Prepared; *cf.* Table II
X	5αP3β20β21	1550	3190	684	Prepared; *cf.* Table II
XI	Δ4P20β21(3)	1640	3215	684	Q1970

* Average G_R-normal value = G_R P20β21 = 681.
** G_R-odd steroid.
*** For L_R value, see Table IV.
§ For ΔG_R value, see Table XIV.

intermediate (average: 22/78) with P17α21(20)-compounds. On the other hand, retention times were higher for P17α20α- than for P17α20β-isomers (*cf.* Tables VII and VIII) as indeed they were for P20α-isomers (*cf.* Tables IV and V), while those of P20α21 and P17α20α21 were shorter than those of their 20β-counterparts (*cf.* Tables X and XI, and Tables XII and XIII). In contrast to previous observations[2,3] on the TLC behaviour of other 20α- and 20β-isomers, P17α20β- and P17α20β21-steroids migrated faster in the present TLC system, *i.e.*, were less polar, than their 20α-counterparts.

TABLE XI

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P20α21

Steroid					*Source*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP20α21	592	2772	659	Calculated; L_R 5βP20α*** + ΔG_R§
II	5αP20α21	638	2805	655	SRC
III	5βP3β20α21	1151	3061	659	Calculated; L_R 5βP3β20α*** + ΔG_R§
IV	5αP3α20α21	1162	3065	664	Calculated; L_R 5αP3α20α*** + ΔG_R§
V	5βP20α21(3)	1173	3069	657	Calculated; L_R 5βP20α(3)*** + ΔG_R§
VI	5βP3α20α21	1203	3080	659	Prepared; *cf.* Table II
VII	5αP20α21(3)	1295	3112	659	Calculated; L_R 5αP20α(3)*** + ΔG_R§
VIII	Δ4P3β20α21	1376	3139	656	Prepared; *cf.* Table II
IX	Δ5P3β20α21	1431	3156	659	Prepared; *cf.* Table II
X	5αP3β20α21	1465	3166	660	Prepared; *cf.* Table II
XI	Δ4P20α21(3)	1556	3192	661	Calculated; L_R Δ4P20α(3)*** + ΔG_R§

* Average G_R-normal value = G_R P20α21 = 658.
** G_R-odd steroid.
*** For L_R value, see Table V.
§ For ΔG_R value, see Table XIV.

TABLE XII

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P17α20β21

Steroid					*Source*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP17α20β21	890	2949	836	Prepared; *cf.* Table II
II	5αP17α20β21	963	2984	834	SRC
III	5βP3β17α20β21	1702	3231	829**	Calculated; L_R 5βP3β17α20β*** + ΔG_R§
IV	5αP3α17α20β21	1741	3241	840	Calculated; L_R 5αP3α17α20β*** + ΔG_R§
V	5βP17α20β21(3)	1738	3240	828**	Calculated; L_R 5βP17α20β(3)*** + ΔG_R§
VI	5βP3α17α20β21	1728	3237	816**	Prepared; *cf.* Table II
VII	5αP17α20β21(3)	1951	3290	837	Calculated: L_R 5αP17α20β(3)*** + ΔG_R§
VIII	Δ4P3β17α20β21	2079	3318	833	SRC; Prepared; *cf.* Table II
IX	Δ5P3β17α20β21	2150	3332	835	Calculated; L_R Δ5P3β17α20β*** + ΔG_R§
X	5αP3β17α20β21	2220	3346	840	Prepared *cf.* Table II
XI	Δ4P17α20β21(3)	2346	3370	839	Calculated; L_R Δ4P17α20β(3)*** + ΔG_R§

* Average G_R-normal value = G_R P17α20β21 = 836.
** G_R-odd steroid.
*** For L_R value, see Table VII.
§ For ΔG_R value, see Table XIV.

In short, the 17α- and 21-hydroxyl groups had opposite effects on the low 20α/20β ratio of products in the RD reduction of (20). In addition, the polarity of the 20α- *vs.* the 20β-isomer was higher in the presence of 17α, and its retention time was shorter in the presence of 21-hydroxyl.

DO. Steroids of groups P17α(20), P21(20), and 17α21(20) reacted abnormally in the presence of a large excess of PTSA catalyst such as was used in previously described dioxolone syntheses (*cf.* refs. 1–3). GLC analysis of the reaction mixtures indicated the formation of thermally unstable products leading to very numerous compounds both of high and low molecular weight, which included little or none of

TABLE XIII

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P17α20α21

Steroid					*Source*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP17α20α21	846	2927	814	Prepared; *cf.* Table II
II	5αP17α20α21	924	2965	815	SRC
III	5βP3β17α20α21	1651	3217	815	Calculated; L_R 5βP3β17α20α*** + ΔG_R***
IV	5αP3α17α20α21	1660	3220	819	Calculated: L_R 5αP3α17α20α** + ΔG_R
V	5βP17α20α21(3)	1680	3225	815	Calculated; L_R 5βP17α20α(3)** + ΔG_R***
VI	5βP3α17α20α21	1728	3236	815	Prepared; *cf.* Table II
VII	5αP17α20α21(3)	1845	3266	813	Calculated; L_R 5αP17α20α(3)** + ΔG_R***
VIII	Δ4P3β17α20α21	1980	3297	814	Prepared; *cf.* Table II
IX	Δ5P3β17α20α21	2041	3310	813	SRC
X	5αP3β17α20α21	2094	3321	815	Prepared; *cf.* Table II
XI	Δ4P17α20α21(3)	2235	3349	818	Calculated; L_R Δ4P17α20α(3)** + ΔG_R***

* Average G_R-normal value = G_R P17α20α21 = 815.
** For L_R value, see Table VIII.
*** For ΔG_R value, see Table XIV.

the expected DO derivatives. Lowering the amount of PTSA to 0.1 mg considerably minimized this effect and led to at least 80% yields of expected dioxolones; the balance consisted mostly of unreacted material.

Shifts of L_R values resulting from DO derivatization, *i.e.*, ΔDO(20) and ΔDO(3,20) values[1–3], were recorded. The averages were 282 $\pm$ 2 and 475 $\pm$ 3 L_R units for steroids of the P17α(20) group, and 196 $\pm$ 2 and 393 $\pm$ 2 units for the steroids of the P21(20) group, respectively. Obtained as a difference between average ΔDO(3,20) and ΔDO(20) values[2,3], the ΔDO(3) values were 193 and 197 for groups P17α(20) and P21(20), respectively.

Decomposition of 17α21(20)-steroids prevented a direct determination of ΔDO values. However, the formation of thermally stable dioxolone derivatives was observed: for example, in the case of 5βP17α21(3,20), the DO(3) and DO(3,20) derivatives were obtained in equal amounts and the following L_R values were recorded: 5βP17α21(20)DO(3): 3550 and 5βP17α21DO(3,20): 3785. Hence the ΔDO(20) value, 235, was obtained by difference. Identification of these derivatives was obtained by RD reduction (2 h) of the reaction mixture which yielded the unaffected DO(3,20) derivative easily separable by TLC from more polar 20β- and 20α-reduction products of the DO(3) dioxolone. Isolated TLC fractions were hydrolyzed yielding the original product from the DO(3,20) derivative and 5βP17α20β(3) and 5βP17α20α(3) from the reduced DO(3) derivative. The ΔDO(3) values of the 20β and 20α compounds were 197 and 200, respectively.

Evidently, the specificity of ΔDO values in the presence of other functional groups[1–3], and consequently, their usefulness in steroid identification[1–3] were confirmed by these data.

Enol acetate. Aside from unreacted P(20)-steroid, or its acetate when a 3α- or 3β-hydroxyl group was present, two major products were invariably obtained (*cf.* Diagrams 1–3), *i.e.*, the two conformational isomeric enol acetates I and II (*cf.* below) in approximately 3/2 ratio, with enol II having the longest retention time. The L_R shift calculated from the L_R value of the initial material or, when a 3α- or 3β-hydroxyl group was present, of its acetate, was consistently 177 $\pm$ 1 L_R units for enol II. The L_R shift corresponding to enol I was 139 $\pm$ 1 for 5βP steroids, and 145 $\pm$ 2 units for all others. These rules also applied to products from 5βP(3,20) and

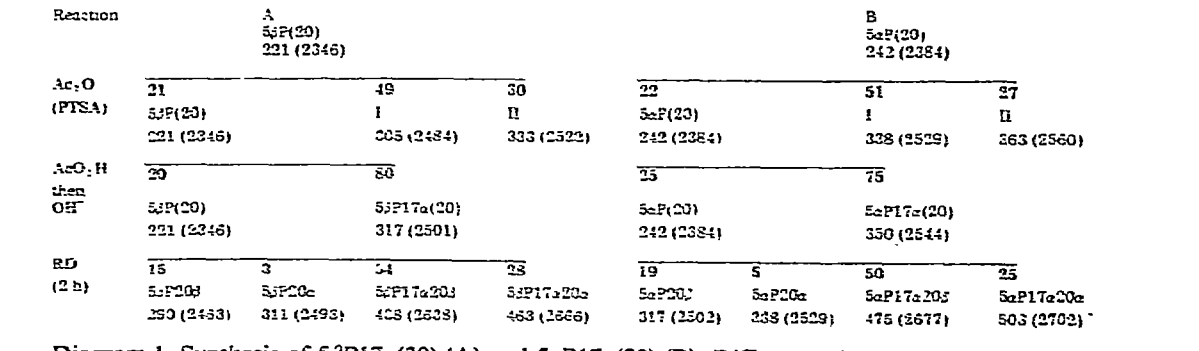

Diagram 1. Synthesis of 5βP17α(20) (A) and 5αP17α(20) (B). Differences in L_R values from starting material to compounds I and II in the first step are 138 and 176 (sequence A) and 139 and 178 (sequence B), respectively.

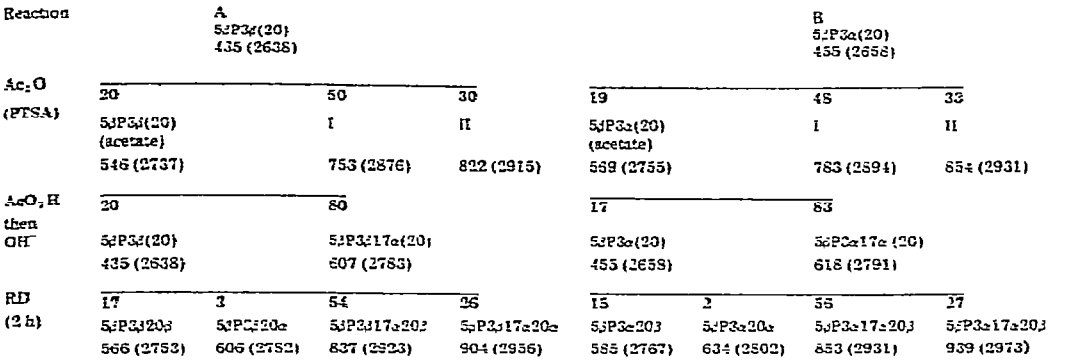

Diagram 2. Synthesis of 5β*P*3β17α(20) (A) and 5β*P*3α17α(20) (B). The acetate of the starting material produced in the first step differs in L_R value from enol acetates I and II by 139 and 178 units in sequence A, and 139 and 176 units in sequence B, respectively. Increases in L_R value resulting from acetylation of the starting material are 99 and 98 units, respectively.

5α*P*(3,20). The L_R shift due to acetylation of 3α or 3β was 73 ± 1 units for 5β*P*-steroids (Diagram 2), and 99 ± 1 units for 5α*P*- or Δ5*P*-compound (Diagram 3).

Because *P*17α(20)-steroids (*cf.* Diagrams 1–3) were obtained in the expected yield from compounds I and II, these were probably the two isomeric enol acetates predicted from Dreiding models. In these models, the 13, 16, 17, 20, and 21 carbon atoms, and the 20-enol oxygen are coplanar, and the C21-methyl group is either *cis* or *trans* in relation to C-16. Presumably, the most compact (lowest energy) isomer was that corresponding to the lowest retention time and highest yield, *i.e.*, compound I. The L_R differences observed were not very large, nor clearly predictable from the models.

In any event, the predictability of the L_R shifts for the twin peaks of enol acetates makes this simple reaction valuable for the identification of P(20)-steroids.

Conversion of enol acetates to 17α-hydroxy(20)-steroids. Treatment of enol acetate mixtures with peracetic acid, followed by saponification of all acetyl groups

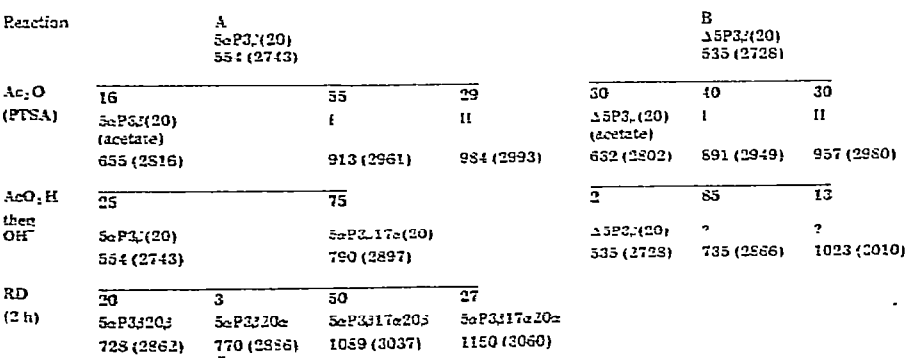

Diagram 3. Synthesis of 5α*P*3β17α(20) (A). Attempted synthesis of Δ15*P*3β17α(20) (B). The L_R value of the acetate of the starting material in the first step and that of compounds I and II are: in sequence A, 145 and 177; in sequence B, 147 and 178 L_R units, respectively. Increases in L_R value resulting from acetylation of the starting material are 73 and 74 units, respectively. Final products in sequence B are discussed in the text.

TABLE XIV

ΔG_R VALUES[a]

Group		*(20)*	*20β*	*20α*	*17α(20β)*	*17α20β*	*17α20α*	*21(20)*	*20β21*	*20α21*	*17α20β21*	*17α20α21*
	G_R	*234*	*353.5*	*380*	*389*	*530*	*554*	*589*	*681*	*658*	*836*	*815*
(20)	234				155[**] (154)			355[***] (353)				
20β	353.5			26.5[§§] (24)		176.5[**] (175)			327.5[***] (328)		482.5 (484)	
20α	380		26.5[§§] (24)				174[**] (174)			278[***] (280)		435 (435)
17α(20)	389	155[**] (154)						200[§] (199)				
17α20β	530		176.5[**] (175)				24[§§] (24)		151[§] (153)		306[***] (309)	
17α20α	554			174[**] (174)		24[§§] (24)				104[§] (106)		261[***] (261)
21(20)	589	355[***] (353)			200[§] (199)							
20β21	681		327.5[***] (328)			151[§] (153)				23[§§] (24)	155[**] (156)	
20α21	658			278[***] (280)			104[§] (106)		23[§§] (24)			156[**] (155)
17α20β21	836		482.5 (484)			306[***] (309)			155[**] (156)			21[§§] (25)
17α20α21	815			435 (435)			261[***] (261)			156[**] (155)	21[§§] (25)	

[a] Average ΔG_R values *vs.* ΔG_R values for 5αP3β compounds shown in parentheses. Average ΔG_R values are differences between normal G_R values listed in row 2 and column 2. The G_R values were taken from footnote[a] in Tables III–XIII. ΔG_R values for 5αP3β compounds were obtained as $\Delta G_R(a,b) = G_R(a) - G_R(b)$ with $G_R(a) > G_R(b)$ where $G_R(a)$ and $G_R(b)$ are G_R values of 5αP3β compounds taken from row X, column 5 in Tables III–XIII.

[**] Contribution of 17α to L_R value of 17α(20), 17α20β, or 17α20α compound.

[***] Contribution of 21-OH to L_R value of 21(20), 20β21, or 20α21 compound.

[§] Difference between contributions of 17α and 21-OH.

[§§] Difference between contributions of 20α and 20β.

(Diagrams 1–3) yielded the expected P17α(20)-steroids as major products besides unmodified initial P(20)-compound, except in the case of Δ5P3β(20) (Diagram 3) and 3-keto steroids (not shown): Although normal enol acetates were produced in these cases (*cf.* above), the final products had abnormal retention times. The identity of normal products of these reactions (Diagrams 1–3) were confirmed by their known retention times (Tables III and IV) and that of their RD reduction products (Tables IV, V, VII, and VIII).

OX. Oxidation by CrO_3 under conditions described in ref. 1 left steroids of group P17α(20) largely intact. Aside from unreacted starting material, insignificant amounts of 5αA3β(17) and 5αA(3,17) were observed by GLC analysis of oxidation products of 5αA3β17α(20), for example.

P21(20)-steroids were completely modified. However, the only peaks observed in the chromatograms corresponded to about 3% of the original material converted to the 17-keto androstane. The main oxidation products, presumably the 20-carboxylic acids, were thermally unstable.

Partial oxidative degradation of the dihydroxyacetone side-chain was observed with P17α-21(20)-steroids. Thus 24% conversion to 5βA(3,17) was obtained with 5βP17α21(3,20). The thermally unstable, unconverted material would not appear in the chromatograms.

These effects were obviously similar to those discussed in ref. 6 obtained with other oxidants.

WK. The effects of WK reagents on P17α(20)-, P21(20)-, and P17α21(20)-steroids were similar in one respect only when observed by GLC: Neither a product corresponding to the simple removal of (20), nor unreacted material could be detected in substantial amounts.

With P17α(20)-steroids, the reaction products arose from the removal of the pregnane side-chain except for minor amounts of fully reduced starting material. With 5βP3β17α(20), for example, the main products were 5βA3β17α, 5βA3β(17), 5βA3β17β, and a compound tentatively identified as Δ16, 5βA3β17β. Conversion to 5βP3β17α was 3% only.

Steroids of group P21(20) kept the pregnane side-chain but lost the 21-hydroxyl group. Products obtained from 5αP21(3,20), for example, were: 5αP, 28.5%; 5αP(20), 27.5%; 5αP20β, 29%; 5αP20α, 9.6%: 5αP3β20β, 3.6%; and 5αP3β20α, 1.8%. No peak corresponded to 5αP21. Not surprisingly, the products of 5βP3α21(20) were 5βP3α, 5βP3α20β, and 5βP3α20α. Again, no 5βP3α21 was formed.

TMS. A comparison of retention times for derivatized and non-derivatized P17α(20)-steroids showed that derivatization of 17α, in 5βP17α(3,20) for example, decreased the retention time. Peaks of derivatized or non-derivatized P17α(20) steroids were fairly symmetrical although unusually broad-based, having about 1.7 times the expected width. The TMS derivatives of the 20α- and 20β-reduction products had normal peaks.

Steroids which featured the 21-hydroxyl group decomposed in the GLC chromatograph unless derivatized; those featuring the dihydroxyacetone side-chain decomposed as derivatives: If enough material was injected, *e.g.*, 200 ng, a broad shallow elevation of the base line would result with a few very broad peaks dominating this background; the total area represented only a fraction of the original material; there was no significant response with 20 ng. However, even low levels of the TMS

derivatives of the 20α- and 20β-reduction products (Tables I, XII, and XIII) gave normal GLC peaks.

HY. Hydrolysis[1] of TMS or dioxolone derivatives was straightforward.

G_R and ΔG_R data (see Table XIV). All P(20) steroids (Table III) and all steroids featuring 20α (Tables V, VIII, XI, and XIII) were G_R-normal. G_R-oddity affected all 5βP(3)- and 5βP3α-steroids featuring 20β (Tables IV, VII, X, and XII), and 5βP3α-steroids featuring (20) (Tables VI and IX). In addition, 5βP3β-steroids featuring 17α were G_R-odd steroids (Tables VI, VII, and XII) unless they featured 20α also. The 15 cases of G_R-oddity found among 121 steroids were much fewer than those previously observed with 11-substituted steroids[1–3].

The L_R values of all G_R-normal steroids were readily calculated by adding M_R, taken from Table I, to the G_R value of the corresponding group. Most calculated values listed in Tables III–XIII were obtained in this way. Calculated and observed values differed but little even when the G_R values of 5αP3β-steroids were used instead. Fortunately, the 5αP3β-steroids were either commercially available or readily prepared by RD reduction (Table II). Errors arising from this convenient procedure reflected small deviations of the G_R values of 5αP3β-steroids from that of the corresponding groups.

G_R values of 5αP3β-steroids were also used to determine ΔG_R values shown in parentheses in Table XIV. Obviously, these did not differ considerably from ΔG_R values obtained as differences of G_R values of group pairs.

As indicated in footnotes** and *** of Table XIV, the relevant ΔG_R values represented contributions to L_R values of functional groups 17α and 21-OH. These contributions varied, often markedly, with the presence of other functional groups in the molecule. The contribution of 21-OH, for example, was 355, 327, 278, 306, and 261 L_R units for P21(20), P20β21, P20α21, P17α20β21, and P17α20α21, respectively.

With ΔG_R values shown in Table XIV, any L_R value listed in Tables III–XIII could be calculated from the L_R value of any *M*-corresponding steroid. Table XII in ref. 3 exemplified this type of operation and demonstrated the precision attainable by the ΔG_R method of calculation. Its application to the present case again demonstrated its reliability, versatility, and practical value in steroid identification.

As previously demonstrated[1–3], the ΔG_R method of calculation applied equally well to G_R-normal and G_R-odd steroids unless either the calculated L_R value, or the reference L_R value was affected by excess oddity. The few cases reported in previous communications were 5βA3α11β17β[1], 5βP3α11β20β[2], and 5βA3α11α17β[3]. Remarkably, cases found in the present data: 5βP3α17α20β, 5βP3α20β21, 5βP3α17α20β21, 5βP3α17α(20), and 5βP3α21(20), involved 5βP3α-steroids also.

The reversal of peak position for 20α- and 20β-isomers due to the presence of a 21-hydroxyl group (*cf.* above) on the one hand, and excess oddity in 5βP3α20β21 and 5βP3α17α20β21, on the other hand, explain the exceptional closeness of L_R values observed for the 20α- and 20β-isomers in both cases (compare values in Tables X and XI, and XII and XIII, respectively). As shown in Table XIV, the normal difference was about 24 L_R units in absolute value.

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