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

V. CONCURRENT SUBSTITUTION IN THE PREGNANE SIDE-CHAIN AND POSITION 11*

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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 the removal of the pregnane side-chain with $NaBiO_3$. GLC chromatograms readily provide information on effects on functional groups at positions 3, 11, 17, 20, and 21 and the retention times of many steroids unavailable from commercial sources. GLC data analysis provides relationships between steroid structure and retention time from which methods for the computation of retention times and steroid identification are designed. The accuracy of these methods is demonstrated.

INTRODUCTION

The fifth in a series of articles[1–4] dealing with reactions and structurally dependent chromatographic properties of steroids, the present communication concerns groups of steroids which include very important corticosteroid hormones and metabolites. The steroids of concern are related to those dealt with in Part IV[4]. Table II, column 4, shows that at least one gas–liquid chromatographic (GLC) property of 17α, 21(20)-steroids is not altered by the introduction of (11) or 11β, *viz.*, their propensity to decompose under GLC conditions. Thermal stability is observed, however, for all other steroids of these groups, including those with a fully reduced side-chain, the 17α, 20α, 21- and 17β, 20β, 21-steroids. Understandably, full reduction of the side-chain will reveal the presence of thermally unstable species. For this and other reasons discussed below, the facile reduction by sodium borohydride (RD)[1] is a very important

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tool in detecting, estimating, and identifying the steroids of concern. Hence ,results obtained by applying this reaction are fully described in Table I*.

In Part IV[4], a study of CrO_3-pyridine reaction with parent steroids substituted in the side-chain had shown that little information useful for the purpose of identification could be derived from the products. Similar results were obtained with the 11-substituted parent compounds. In contrast, sodium bismuthate oxidation ($NaBiO_3$) produced high yields, generally of a single product characteristic of the original steroid[5].

The present article reports in Table II the results of an extensive investigation of this reaction with all types of steroids substituted in the side-chain, including those not substituted at position 11. The normal products with this last category of steroids are described in Table XIII. GLC data on the normal products of (11)- and 11β-substituted steroids are given in Tables XIV and XV, respectively.

In Part IV[4], the normalcy[1] of retention time t'_{NR}, of steroids substituted in the side-chain, though not at position 11, was shown to be a general feature. For these compounds, the L_R value expressed by

$$L_R = 10^3 \times \log t'_{NR} \tag{1}$$

is the sum of two constants

$$L_R = M_R + G_R \tag{2}$$

where G_R is characteristic of the group to which the steroid belongs, and M_R a value characteristic of the structure of ring A. M_R values are listed in Table I of ref. 4. Thus the M_R value for steroids with the same ring A or M-features is identical by definition. Hence the difference ΔG_R (a,b) of the L_R values, L_R (a) and L_R (b), for M-corresponding steroids in groups (a) and (b), is given by the difference of the corresponding G_R values, as follows from eqn. 2

$$\Delta G_R \text{ (a,b)} = L_R \text{ (a)} - L_R \text{ (b)} = G_R \text{ (a)} - G_R \text{ (b)} \tag{3}$$

As shown in refs. 1–3, deviations of G_R indicating oddity occur for certain steroids of the same group as a result of 11-substitution. However, it was shown also that such deviations are identical in sign and size for M-corresponding steroids, and that eqn. 3, therefore, is still applicable to odd steroids[1–4]. Hence, the L_R value of any steroid, L_R (a), can be calculated from the known value L_R (b) of an M-corresponding steroid featuring the same substitution at position 11 by

$$L_R \text{ (a)} = L_R \text{ (b)} + \Delta G_R \text{ (a,b)} \tag{4}$$

Here ΔG_R (a,b) is characteristic of groups (a) and (b) and readily calculable by eqn. 3 given any pair of L_R values L_R (a) and L_R (b) for M-corresponding steroids of these groups (Table XVI).

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* In the tables, under source, a letter followed by four digits indicates the catalogue number of Steraloids Inc., P.O. Box 127, Pawling, N.Y. 12564, U.S.A. Steroids provided by the Steroid Standard Collection are indicated by SRC; see Acknowledgements.

The present GLC data on 11-substituted steroids, together with similar data from Parts I[1], II[2], and IV[4], were used to test the accuracy of L_R values calculated by eqn. 4 (Table XVIII).

As shown in the discussion, the wide range of applicability and the reliability of this method of calculation thus demonstrated allow the retention time of steroids of given structure to be accurately predicted. The use of this method of calculation, together with the application of selected reactions whose products are characteristic of the given structure therefore permits the unequivocal identification of a steroid to be made even when a sample of the compound is unavailable. The advantages of this novel approach for the systematic analysis of steroids are discussed.

EXPERIMENTAL

Reactions

Procedures used for the RD and the trimethylsilyl (TMS) derivatization of hydroxyl groups have been described in detail[1].

The procedure used for sodium bismuthate ($NaBiO_3$) oxidation was as follows: From 0 to 25 μg of steroid were placed in a 1-ml volumetric-flask (Corning tube No. 5640) by adding with a microsyringe the required volume of solution in methanol and evaporating the solvent under nitrogen. To the contents were added successively, 15 $\pm$ 1 mg of $NaBiO_3$ (Analar; BDH, Toronto, Canada), a 1/8 in. $\times$ 1/2 in. PTFE covered micro-magnet (Fisher No. 9-312-102), and 120 μl of 50% (w/v) aqueous acetic acid. The tube was placed in a holder over a strong magnetic stirrer (Gyrathron, Bronwill Scientific Model 25210) adjusted to 350 rpm.

The holder consisted of two 10 cm diameter, 20 mm thick disks of styrofoam glued together face to face after one of the disks had been punched with a No. 4 cork borer to produce a series of 8-mm holes right through and 20 mm from its center. Up to ten tubes held in these holes in a vertical position could be processed simultaneously.

After 30 min of stirring at room temperature, 120 μl of 25% aqueous NaOH were added dropwise to the tube, which was stirred for 1 min. Using a 3 cm long bar magnet, the micro-magnet was made to slide up the side to the neck of the tube where it was seized at one end with tweezers, and held just above the mouth of the tube. It was washed in this position with 600 μl of benzene added dropwise, the washings being collected in the tube. After 1 min of vigorous stirring with a vortex mixer, the mixture was centrifuged for 1 min. The clear supernatant (solvent) was carefully removed with a 1-ml syringe (point style No. 3, gauge 22 needle). The extraction with 600 μl of benzene was repeated three times. The combined extracts (2.4 ml) were brought, either by dilution or evaporation depending on the concentration, to such a volume that a 50-μl sample contained enough material for GLC analysis. The sample was evaporated to dryness under nitrogen and submitted to TMS derivatization.

Gas–liquid and thin-layer chromatography

Both gas–liquid and thin-layer chromatography (TLC) methods were used as previously described[1–4]. All t'_{NR} values were obtained with steroids or steroid mixtures submitted to TMS derivatization.

DISCUSSION

Reactions

Reduction by $NaBH_4$. RD is as shown in Table I. The behaviour of steroids substituted at position 11 paralleled that of the parent steroids described in Table II of Part IV[4]. High yields of 20α and 20β products giving excellent GLC chromatograms after TMS derivatization were observed even when the original (20)-steroid was thermally unstable and did not give a GLC peak. While the 20β product again was the major one in all cases, the 20α/20β ratios did not differ as sharply among different groups as they did with the corresponding groups of parent steroids[4]. However, the 21-hydroxyl group again reversed the order of appearance of the GLC peaks of the 20α- and 20β steroids, as shown by the t'_{NR} values listed in Tables V and VI, VIII and IX, and XI and XII.

TABLE I

REDUCTION BY $NaBH_4$ (2 h) OF 11-SUBSTITUTED 20-KETONES

Starting material				*Normal product**	
Abbreviation	*Source*	*GLC properties*		*20α/20β ratio*	*GLC properties*
		t'_{NR}	L_R		
5βP3α17α(11,20)	P6200	783	2894	17/83	*cf.* Tables V (β)
5αP17α(3,11,20)	P4100	905	2956	18/82	and VI (α)
Δ5P3β17α(11,20)	SRC	989	2995	17/83	
Δ4P11β17α(3,20)	Q1520, SRC	1417	3151	19/81	
				Av. 18/82	
5αP3α21(11,20)	P2200	1244	3095	15/85	*cf.* Tables VIII (β)
5βP3α21(11,20)	P6240	1178	3071	13/87	and IX (α)
5αP3β21(11,20)	P2230	1648	3217	13/87	
Δ4P21(3,11,20)	Q3690	1647	3217	18/82	
5βP11β21(3,20)	P6270	1510	3179	15/85	
5αP3β11β21(20)	P5400	2059	3314	15/85	
Δ4P11β21(3,20)	Q1550	2250	3352	15/85	
				Av. 15/85	
5βP17α21(3,11,20)	P7100	decomposes		23/77	*cf.* Tables XI (β)
5βP3α17α21(11,20)	P9550	decomposes		23/77	and XII (α)
5αP3β17α21(11,20)	P5200	decomposes		18/82	
Δ4P17α21(3,11,20)	Q2500	decomposes		19/82	
5βP3α11β17α21(20)	P9050	decomposes		17/83	
Δ5P3β11β17α21(20)	Q5750	decomposes		17/83	
Δ4P11β17α21(3,20)	Q3880	decomposes		20/80	
				Av. 20/80	

* While the RD 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. For 30-min reductions leading to compounds still featuring (11), *cf.* text.

As always[1–4], the 3-keto group in 5βP(3)-steroids was converted to 3α and to 3β in all others. The 11-keto group yielded 11β exclusively after 2 h reduction[1,2]. After 30 min, only a fraction of (11) was reduced, while (20) was completely reduced to 20α and 20β[1,2]. The preparation of (11)-steroids completely reduced in the side-chain was therefore possible (see Tables III, IV, VII, and X).

TABLE II

MOLE PER CENT COMPOSITION, *C*, AND TOTAL PER CENT RECOVERY, *R*, OF PRODUCTS FROM $NaBiO_3$ OXIDATION OF STEROIDS SUBSTITUTED IN THE PREGNANE SIDE-CHAIN

*Starting material**				*Normal product***	
No.	*Abbreviation*	*Source*	*GLC properties*	*C (in brackets) (mole %)*	*R (%)*
1	5βP3α17α20β	P9480	*cf.* Table VII of ref. 4	[0.8] unchanged, [12.7] 5βP3α(20), [21.8] 5βA3α(17), and [64.6] 5βP3α20β	84
2	5βP3α17α20α	P9450	*cf.* Table VIII of ref. 4	[6] unchanged, [93] 5βA3α(17)	80
3	Δ5P3β17α20α	SRC	*cf.* Table VIII of ref. 4	[25] unchanged, [40] Δ5A3β(17), [35] 5,6-epoxy-A3β(17)	65
4	Δ5P3β17α20β	Q5890	*cf.* Table VII of ref. 4	[25.5] unchanged, [35.5] Δ5A3β(17), [38.5] 5,6-epoxy-A3β(17)	70
5	Δ4P17α20α(3)	Q1820	*cf.* Table VIII of ref. 4	[25] unchanged, [75] Δ4A(3,17)	72
6	Δ4P17α20β(3)	Q1850	*cf.* Table VII of ref. 4	[19] unchanged, [81] Δ4A(3,17)	70
7	5βP17α21(3,20)	P6300	decomposes	[95] 5βA(3,17)	75
8	5αP17α21(3,20)	P2320	decomposes	[97] 5αA(3,17)	66
9	Δ4P17α21(3,20)	Q1610	decomposes	[97] Δ4A(3,17)	61
10	5αP17α20β21	SRC	*cf.* Table XII of ref. 4	[96] 5αA(17)	65
11	Δ5P3β17α20α21	SRC	*cf.* Table XIII of ref. 4	[75] Δ5A3β(17), [25] 5,6-epoxy-A3β(17)	65
12	Δ4P17α20β21(3)	Q4080	*cf.* Table XII of ref. 4	[97] Δ4A(3,17)	49
13	5αP17α(3,11,20)	P4100	*cf.* Table I	[11] unchanged, [89] 5αA(3,17)	63
14	5βP17α21(3,11,20)	P7100	decomposes	[92] 5βA(3,11,17)	56
15	5βP3α17α21(11,20)	P9550	decomposes	[95] 5βA3α(11,17)	59
16	5αP3β17α21(11,20)	P5200	decomposes	[97] 5αA3β(11,17)	61
17	Δ4P17α21(3,11,20)	Q2500	decomposes	[97] Δ4A(3,11,17)	53
18	5βP3α17α20β21(11)	P9200	*cf.* Table X	[96] 5βA3α(11,17)	67
19	5βP3α11β17α21(20)	P9050	decomposes	[97] 5βA3α11β(17)	68
20	5αP11β17α21(3,20)	P5250	decomposes	[98] 5αA11β(3,17)	49
21	Δ5P3β11β17α21(20)	Q5750	decomposes	[66] Δ5A3β11β(17), [27] 5,6-epoxy-Δ5A3β11β(17)	48
22	Δ4P11β17α21(3,20)	Q3880	decomposes	[97] Δ4A11β(3,17)	60
23	5βP3β11β17α20β21	P8620	*cf.* Table XI	[96.5] 5βA3β11β(17), [4.5] 5βP3β(11,17)	64
24	5βP3α11β17α20β21	P8590	*cf.* Table XI	[85] 5βA3α11β(17), [15] 5βA3α(11,17)	65
25	5αP3β11β17α20β21	P4350	*cf.* Table XI	[97] 5αA3β11β(17)	70
26	Δ4P11β17α20β21(3)	Q3790	*cf.* Table XI	[96] Δ4A11β(3,17)	52
27	Δ4P11β17α20α21(3)	Q3760	*cf.* Table XII	[98] Δ4A11β(3,17)	54

* All starting materials listed were available from outside sources; prepared compounds were also used (*cf.* text).

** The L_R values of most products are listed in Tables XIII–XV.

While a complete RD revealed the presence of 17α21(20)-steroids unaccountable by direct GLC (*cf.* above), (3) and (11) groups were also reduced in the process[1–4]. The products therefore gave a clue as to the presence of substitution at positions 3 and/or 11, but none as to the original nature of these substitutions. This and other structural problems were readily solved by $NaBiO_3$ oxidation.

$NaBiO_3$ oxidation. In 1953, Brooks and Norymberski[5] showed that $NaBiO_3$ oxidation of steroids substituted in the pregnane side-chain produced high yields of fragments characteristic of their structure and proposed the analysis of these products as a means of estimating these steroids. While the estimation of corticosteroids by quantification of the formaldehyde generated by this reaction has proved reliable[6], later work by Breuer and Nocke[7] has revealed complications precluding the use of the 17-ketosteroid products for the quantitative estimation of steroids substituted in the side-chain.

The present, systematic investigation of the reaction products has shown, however, that their analysis by GLC afforded a reliable tool for the identification of the original steroids. Indeed, with the sole exception of 20β-pregnanetriol (No. 1), all steroids listed in Table II yielded the readily identifiable parent 17-ketoandrostane as a major, if not the sole product of $NaBiO_3$ oxidation which was demonstrable by

TABLE III

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P17α20β(11)

Steroid					*Source(s)*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP17α20β(11)	564	2751	638**	Calculated; L_R 5βP20β(11)*** + ΔG_R§
II	5αP17α20β(11)	632	2801	651	Calculated; L_R 5αP20β(11)*** + ΔG_R§
III	5βP3β17α20β(11)	1130	3053	651	Calculated; L_R 5βP3β20β(11)*** + ΔG_R§
IV	5αP3α17α20β(11)	1132	3054	653	Calculated; L_R 5αP3α20β(11)*** + ΔG_R§
V	5βP17α20β(3,11)	1099	3041	638**	Calculated; L_R 5βP20β(3,11)*** + ΔG_R§
VI	5βP3α17α20β(11)	1114	3047	626**	Prepared; 30 min RD 5βP3α17α(11,20)
VII	5αP17α20β(3,11)	1250	3097	644**	Calculated; L_R 5αP20β(3,11)*** + ΔG_R§
VIII	Δ4P3β17α20β(11)	1321	3121	638**	Calculated; L_R Δ4P3β20β(11)*** + ΔG_R§
IX	Δ5P3β17α20β(11)	1396	3145	648	Prepared; 30 min RD Δ5P3β17α(11,20)
X	5αP3β17α20β(11)	1459	3164	658	Prepared; 30 min RD 5αP17α(3,11,20)
XI	Δ4P17α20β(3,11)	1422	3153	622**	Calculated; L_R Δ4P20β(3,11)*** + ΔG_R§

* Average G_R-normal value = G_R P17α20β(11) = 654.

** G_R-odd steroid.

*** For L_R value, see Table IX of ref. 2.

§ ΔG_R = 141; *cf.* Table XVI.

TABLE IV

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P17α20α(11)

Steroid					*Source(s)*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP17α20α(11)	624	2795	682**	Calculated; L_R 5βP20α(11)*** + ΔG_R§
II	5αP17α20α(11)	685	2836	686	Calculated; L_R 5αP20α(11)*** + ΔG_R§
III	5βP3β17α20α(11)	1245	3095	693	Calculated; L_R 5βP3β20α(11)*** + ΔG_R§
IV	5αP3α17α20α(11)	1236	3092	691	Calculated; L_R 5αP3α20α(11)*** + ΔG_R§
V	5βP17α20α(3,11)	1213	3084	672**	Calculated; L_R 5βP20α(3,11)*** + ΔG_R§
VI	5βP3α17α20α(11)	1239	3093	672**	Prepared; 30 min RD 5βP3α17α(11,20)
VII	5αP17α20α(3,11)	1358	3133	680**	Calculated; L_R 5αP20α(3,11)*** + ΔG_R§
VIII	Δ4P3β17α20α(11)	1426	3154	669**	Calculated; L_R Δ4P3β20α(11)*** + ΔG_R§
IX	Δ5P3β17α20α(11)	1518	3181	684	Prepared; 30 min RD Δ5P3β17α(11,20)
X	5αP3β17α20α(11)	1566	3195	689	Prepared; 30 min RD 5αP17α(3,11,20)
XI	Δ4P17α20α(3,11)	1542	3188	657**	Calculated; L_R Δ4P20α(3,11)*** + ΔG_R§

* Average G_R-normal value = G_R P17α20α(11) = 688.
** G_R-odd steroid.
*** For L_R value, see Table X of ref. 2.
§ ΔG_R = 166; *cf.* Table XVI.

GLC. The important point is, of course, that this product possesses all structural features not included in the side-chain of the original steroid. Together with the TLC and GLC data on the original steroid, this information constitutes a basis for unequivocal identification.

The case of 20β-pregnanetriol which yields the parent 20β-diol as major product is remarkable. No other triol, nor any of the corresponding (11)- or 11β-substituted steroids, including the 5βP3α20β compounds No. 18 and No. 24 showed similar behaviour.

The Δ53β-steroids, in addition to the normal 17-keto products, yielded important amounts of the corresponding 5,6-epoxy derivatives (*cf.* compounds Nos. 3, 4, 11, and 21 in Table II) which gave additional evidence for the Δ5P3β structure. Remarkably, the L_R values of the 17-keto and 5,6-epoxy-17-keto products always differed by 140 ± 1 L_R units. Identical 5,6-epoxides were obtained by $NaBiO_3$ oxidation of the Δ53β(17)-steroids listed in Tables XIII–XV. For example, 90% of Δ5A3β (17) was converted under standard conditions to the 5,6-epoxy derivative in 4 h.

While the 21(20)-, 20β21-, and 20α21-steroids yielded normal GLC peaks (*cf.* Table I of Part IV[4] and the present article), their $NaBiO_3$ oxidation products, as indeed their CrO_3 oxidation products[4] gave no GLC peaks. Presumably the 20-

TABLE V

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P11β17α20β

Steroid					*Source(s)*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP11β17α20β	650	2813	700**	Calculated; L_R 5βP11β20β*** + ΔG_R§
II	5αP11β17α20β	736	2867	717	Calculated; L_R 5αP11β20β*** + ΔG_R§
III	5βP3β11β17α20β	1285	3109	707**	Calculated; L_R 5βP3β11β20β*** + ΔG_R§
IV	5αP3α11β17α20β	1288	3110	709**	Calculated; L_R 5αP3α11β20β*** + ΔG_R§
V	5βP11β17α20β(3)	1312	3118	706**	Calculated; L_R 5βP11β20β(3)*** + ΔG_R§
VI	5βP3α11β17α20β	1247	3096	675**	P8750; prepared 2 h RD P6200; *cf.* Table I
VII	5αP11β17α20β(3)	1507	3178	725	Calculated; L_R 5αP11β20β(3)*** + ΔG_R§
VIII	Δ4P3β11β17α20β	1560	3193	710**	Prepared; 2 h RD Δ4P17α20β(3,11); *cf.* Table I
IX	Δ5P3β11β17α20β	1656	3219	722	Prepared; 2 h RD Δ5P3β17α(11,20); *cf.* Table I
X	5αP3β11β17α20β	1702	3231	725	Prepared; 2 h RD 5αP17α(3,11,20); *cf.* Table I
XI	Δ4P11β17α20β(3)	1786	3252	721	Calculated; L_R Δ4P11β20β(3)*** + ΔG_R§

* Average G_R-normal value = G_R P11β17α20β = 723.
** G_R-odd steroid.
*** For L_R value, see Table XI of ref. 2.
§ ΔG_R = 168; *cf.* Table XVI.

carboxylic acid arising from 21(20)-steroids[5] is not extracted from the neutralized reaction mixture and the 17-aldehydic products from 20β21- and 20α21-steroids[5] would be thermally unstable.

The 21(20)-, 20α21-, and 20β21-steroids, therefore, are characterized by the disappearance of their GLC peak after $NaBiO_3$ treatment and the absence of any product demonstrable by GLC. This property confirms a tentative identity based on TLC and GLC data of the original steroid and its RD products.

The characteristic property of 17α(20)-steroids is that they are unaffected by $NaBiO_3$ (ref. 5).

Because of appreciable differences in reactivity towards $NaBiO_3$, the standard conditions described above were selected as a satisfactory compromise ensuring good, roughly uniform yields in all cases.

Yields increased for the least reactive, but decreased for the most reactive steroids when the reaction time was extended from 30 min to 45 min, all other conditions remaining as previously described. Beyond 1 h, a general deterioration of recoveries was observed. A time-dependent decrease in recovery was also demonstrable when the oxidation procedure was applied directly to the 17-keto oxidation products listed in Tables XIII–XV. However, because the recoveries of these compounds still

TABLE VI

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P11β17α20α

Steroid					*Source(s)*
M	*Abbreviation*	t'_{NR}	G_R*		
I	5βP11β17α20α	718	2856	743**	Calculated; L_R 5βP11β20α*** + ΔG_R§
II	5αP11β17α20α	800	2903	753	Calculated; L_R 5αP11β20α*** + ΔG_R§
III	5βP3β11β17α20α	1413	3150	732**	Calculated; L_R 5βP3β11β20α*** + ΔG_R§
IV	5αP3α11β17α20α	1409	3149	748**	Calculated; L_R 5αP3α11β20α*** + ΔG_R§
V	5βP11β17α20α(3)	1452	3162	750**	Calculated; L_R 5βP11β20α(3)*** + ΔG_R§
VI	5βP3α11β17α20α	1416	3151	730**	SRC; prepared 2 h RD P6200; *cf.* Table I
VII	5αP11β17α20α(3)	1641	3215	762	Calculated; L_R 5αP11β20α(3)*** + ΔG_R§
VIII	Δ4P3β11β17α20α	1683	3226	743**	Prepared; 2 h RD Δ4P17α20β(3,11); *cf.* Table I
IX	Δ5P3β11β17α20α	1782	3251	754	Prepared; 2 h RD Δ5P3β17α(11,20); *cf.* Table I
X	5αP3β11β17α20α	1841	3265	759	Prepared; 2 h RD 5αP17α(3,11,20); *cf.* Table I
XI	Δ4P11β17α20α(3)	1941	3288	757	Calculated; L_R Δ4P11β20α(3)*** + ΔG_R§

* Average G_R-normal value = G_R P11β17α20α = 757.
** G_R-odd steroid.
*** For L_R value, see Table XII of ref. 2.
§ ΔG_R = 158, *cf.* Table XVI.

exceeded 90% after 30 min, and because at least 3 h of exposure to the reagents was necessary to bring these recoveries down to the levels R listed in Table II, losses indicated by R could not possibly arise exclusively from the degradation and extractive losses of the 17-keto products. Rather, they resulted mainly from a reaction involving a more reactive precursor of these compounds, possibly through fusion at the level of C-17 of two precursor molecules. The extreme broadness and late emergence of the GLC peak would make a dimer-size compound undetectable in small amounts. The apparent degradation of the 17-keto compound probably occurs via the formation of this labile precursor from the 17-keto compound itself. The existence of an equilibrium between precursor and 17-keto compound largely favouring the latter would explain the comparatively slow decrease in recovery observed with the 17-keto compound as such.

All other conditions being as described above, yields increased markedly when the amount of $NaBiO_3$ was increased up to 10 mg, then slowly up to 15 mg. No further beneficial effect was observed up to 25 mg, possibly because larger quantities of $NaBiO_3$ made stirring and extraction of the reaction mixture less effective. Likewise stirring increased the reaction rate up to 250–300 rpm only.

The effect of varying the acetic acid concentration from 25 to 80% was not very

TABLE VII

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P20β21(11)

Steroid					*Source(s)*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP20β21(11)	867	2938	825**	Calculated; L_R 5βP20β(11)*** + ΔG_R§
II	5αP20β21(11)	973	2988	838	Calculated; L_R 5αP20β(11)*** + ΔG_R§
III	5βP3β20β21(11)	1738	3240	838	Calculated; L_R 5βP3β20β(11)*** + ΔG_R§
IV	5αP3α20β21(11)	1738	3240	839	Prepared; 30 min RD 5αP3α21(11,20)
V	5βP20β21(3,11)	1690	3228	816**	Calculated; L_R 5βP20β(3,11)*** + ΔG_R§
VI	5βP3α20β21(11)	1629	3212	791**	Prepared; 30 min RD 5βP3α21(11,20)
VII	5αP20β21(3,11)	1923	3284	831**	Calculated; L_R 5αP20β(3,11)*** + ΔG_R§
VIII	Δ4P3β20β21(11)	2032	3308	825**	Prepared; 30 min RD Δ4P21(3,11,20)
IX	Δ5P3β20β21(11)	2163	3335	838	Calculated; L_R Δ5P3β20β(11)*** + ΔG_R§
X	5αP3β20β21(11)	2228	3348	842	Prepared; 30 min RD 5αP3β21(11,20)
XI	Δ4P20β21(3,11)	2188	3340	809**	Calculated; L_R Δ4P20β(3,11)*** + ΔG_R§

* Average G_R-normal value = G_R P20β21(11) = 839.
** G_R-odd steroid.
*** For L_R value, see Table IX of ref. 2.
§ ΔG_R = 328; *cf.* Table XVI.

marked, the optimal plateau stretching from 45 to 65%. In all cases, the volume of 25% (w/v) NaOH added to the reaction mixture provided 75% neutralization[5]. The density of the neutralized reaction mixture being much higher than that of the solvent, a clean centrifugal separation was obtained, but vigorous stirring and several extractions were needed for highest recovery. The volume of benzene used had to be kept at or below 600 μl, and the total volume below the 1-ml mark to prevent losses due to splashing while stirring with the vortex mixer.

No beneficial effect accrued from working under nitrogen or in complete darkness.[5]

The recoveries listed in Table II were obtained with 10-μg samples of steroid. A slow decrease down to about half of these values was observed as the amount of steroid was lowered to 50 ng. The $NaBiO_3$ test was still sensitive, however, because the products gave narrow peaks in the early section of the GLC chromatogram. For example, 1 ng of androsterone gave a 50-mm peak at full sensitivity. The sensitivity of the test was lowest with Δ4A (3,17), Δ4A (3,11,17), and Δ4A11β (3,17) because corresponding peaks had a tendency to flatten and shift upscale at low level[1,8]. Improved sensitivity was obtained in this case by analyzing the Δ4A3β17β compounds obtained by RD of the products or the Δ4A3β(17) products of the RD-subjected steroids.

TABLE VIII

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P11β20β21

Steroid					*Source(s)*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP11β20β21	940	2973	860**	Calculated; L_R 5βP11β20β*** + ΔG_R§
II	5αP11β20β21	1064	3027	877	Calculated; L_R 5αP11β20β*** + ΔG_R§
III	5βP3β11β20β21	1858	3269	867**	Calculated; L_R 5βP3β11β20β*** + ΔG_R§
IV	5αP3α11β20β21	1870	3272	870**	Prepared; 2 h RD 5αP3α21(11,20); *cf.* Table I
V	5βP11β20β21(3)	1897	3278	866**	Calculated; L_R 5βP11β20β(3)*** + ΔG_R§
VI	5βP3α11β20β21	1766	3247	826**	Prepared; 2 h RD 5βP3α21(11,20); *cf.* Table I
VII	5αP11β20β21(3)	2178	3338	880	Calculated; L_R 5αP11β20β(3)*** + ΔG_R§
VIII	Δ4P3β11β20β21	2254	3353	870**	Prepared; 2 h RD Δ4P21(3,11,20) + ΔG_R§
IX	Δ5P3β11β20β21	2388	3378	881	Calculated; L_R Δ5P3β11β20β + ΔG_R§
X	5αP3β11β20β21	2460	3391	885	Prepared; 30 min RD 5αP3β21(11,20); *cf.* Table I; 2 h RD 5αP3β11β21(20); *cf.* Table I
XI	Δ4P11β20β21(3)	2582	3412	883	Calculated; L_R Δ4P11β20β*** + ΔG_R§

* Average G_R-normal value = G_R P11β20β21 = 881.
** G_R-odd steroid.
*** For L_R value, see Table XI of ref. 2.
§ ΔG_R = 328; *cf.* Table XVI.

G_R and ΔG_R data

The G_R data in Tables III, IV, VII, and X show that the pattern of G_R-odd steroids in groups featuring (11) is characteristic and different from the characteristic pattern in groups featuring 11β (*cf.* Tables V, VI, VIII, IX and XII). A comparison with data previously discussed[1,2] shows that, indeed, the patterns corresponding to (11) and 11β are unique.

Table XVI shows ΔG_R values calculated by eqn. 3 for groups listed in column 1 and the groups indicated in rows — from G_R values shown between parentheses under group designations. The ΔG_R values listed in the column are the differences between G_R values of group pairs to which 226 was added with groups of the androstane series (*cf.* eqn. 17 of ref. 1).

The mean differences, ΔG_R of L_R values for *M*-corresponding 20α and 20β isomers, including those previously observed[2–4], are presented in Table XVII for comparison. Obviously, ΔG_R varies from group to group. While the introduction of (11) or 11β exerts an unpredictable influence, it does not affect the reversal in order of

TABLE IX

VALUES OF L_R AND G_R, AND SOURCE OF STEROIDS OF GROUP P11β20α21

Steroid					*Source(s)*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP11β20α21	912	2960	847**	Calculated; L_R 5βP11β20α*** + ΔG_R§
II	5αP11β20α21	1016	3007	857	Calculated; L_R 5αP11β20α*** + ΔG_R§
III	5βP3β11β20α21	1799	3255	853**	Calculated; L_R 5βP3β11β20α*** + ΔG_R§
IV	5αP3α11β20α21	1795	3254	853**	Prepared; 2 h RD 5αP3α21(11,20); *cf.* Table I
V	5βP11β20α21(3)	1845	3266	854**	Calculated; L_R 5βP11β20α(3)*** + ΔG_R§
VI	5βP3α11β20α21	1656	3219	798**	Prepared; 2 h RD 5βP3α21(11,20); *cf.* Table I
VII	5αP11β20α21(3)	2084	3319	866	Calculated; L_R 5αP11β20α(3)*** + ΔG_R§
VIII	Δ4P3β11β20α21	2133	3329	846**	Prepared; 2 h RD Δ4P21(3,11,20)***; *cf.* Table I
IX	Δ5P3β11β20α21	2270	3356	859	Calculated; L_R Δ5P3β11β20α*** + ΔG_R§
X	5αP3β11β20α21	2355	3372	866	Prepared; 30 min RD 5αP3β21(11,20); *cf.* Table I; 2 h RD 5αP3β11β21(20); *cf.* Table I
XI	Δ4P11β20α21(3)	2472	3393	862	Calculated; L_R Δ4P11β20α(3)*** + ΔG_R§

* Average G_R-normal value = G_R P11β20α21 = 862.
** G_R-odd steroid.
*** For L_R value, see Table XII of ref. 2.
§ ΔG_R = 262; *cf.* Table XVI.

appearance of peaks indicated by a negative ΔG_R value for groups which feature 21-OH.

Using eqn. 4, the L_R values of steroids listed in column 1 of Table XVIII were calculated, and the errors between calculated and observed values were entered in columns below the designations of the relevant groups. Because only errors obtained from experimental L_R values are significant in this test, steroids for which a complete set of significant values was unavailable were not included in column 1. Steroids of particular *M*-configurations, therefore, are not represented by these data. It should be noted, however, that observed L_R data on steroids featuring these *M*-configurations and belonging to all groups designated in columns 2–7 of Table XVIII were used in refs. 1 and 2 to demonstrate the validity of eqn. 4 by the same test.

It is evident that very small errors, never exceeding ± 3 L_R units, resulted from the use of eqn. 4 for L_R values calculations, except in the case of 5βP3α-steroids, for which the errors were large[1,2]. However, errors observed with the 5β3α-steroids corresponding to columns 2–5 were so uniform (± 2) that the following corrections could

TABLE X

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P17α20β21(11)

Steroid					*Source(s)*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP17α20β21(11)	1167	3067	956**	Calculated; L_R 5βP20β(11)*** + ΔG_R§
II	5αP17α20β21(11)	1309	3117	967	Calculated; L_R 5αP20β(11)*** + ΔG_R§
III	5βP3β17α20β21(11)	2339	3370	969	Calculated; L_R 5βP3β20β(11)*** + ΔG_R§
IV	5αP3α17α20β21(11)	2344	3370	969	Calculated; L_R 5αP3α20β(11)*** + ΔG_R§
V	5βP17α20β21(3,11)	2275	3357	945**	Calculated; L_R 5βP20β(3,11)*** + ΔG_R§
VI	5βP3α17α20β21(11)	2218	3346	925**	P9200; prepared 30 min RD P7100 and P9550
VII	5αP17α20β21(3,11)	2588	3413	960**	Calculated; L_R 5αP20β(3,11)*** + ΔG_R§
VIII	Δ4P3β17α20β21(11)	2733	3436	953**	Prepared; 30 min RD Δ4P17α21(3,11,20)
IX	Δ5P3β17α20β21(11)	2911	3464	967	Calculated; L_R Δ5P3β20β(11)*** + ΔG_R§
X	5αP3β17α20β21(11)	3010	3478	972	Prepared; 30 min RD 5αP3β17α21(11,20)
XI	Δ4P17α20β21(3,11)	2944	3469	938**	Calculated; L_R Δ4P20β(3,11)*** + ΔG_R§

* Average G_R-normal value = G_R P17α20β21(11) = 969.

** G_R-odd steroid.

*** For L_R value, see Table IX of ref. 2.

§ ΔG_R = 457; *cf.* Table XVI.

be used successfully: −28 L_R units for (11)-featuring steroids, −27 L_R units for 11β20β-steroids, and −19 with 11β20α-steroids. Thus, at least for these 5β3α-steroids, the extent of excess oddity[1,2] also is predictable. Obviously, this is not so for the other 5β3α-steroids. However, samples of 5β3α-steroids are among the most easily obtainable, either as such or by RD of appropriate keto-steroids.

As the above results show, the L_R values of heavily substituted steroids can be accurately calculated from the L_R value of any less substituted, *M*-corresponding steroid of a related group, and vice versa. These results therefore confirm the principle of constancy of oddity previously enunciated[2], and the reliability, and versatility of a method of L_R value calculation based on eqn. 4. Conversely, this method allows a preliminary determination of structure to be made from retention time data.

Ambiguities that may arise in cases when two or more steroids of different structure have the same, or nearly the same retention time are often dispelled by the TLC data. In our systematic analysis of steroids, TLC is used as a preliminary separation step to obtain several fractions within precisely determined boundaries[1]. Hence, aside from making a subsequent GLC separation of the fraction components possible, this procedure also supplies TLC data on these components which may be decisive in the identification process. The corticosteroids, for example, are found in neatly

TABLE XI

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P11β17α20β21

Steroid					*Source(s)*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP11β17α20β21	1327	3123	1010**	Calculated; L_R 5βP11β20β*** + ΔG_R§
II	5αP11β17α20β21	1503	3177	1027	Calculated; L_R 5αP11β20β*** + ΔG_R§
III	5βP3β11β17α20β21	2600	3415	1013**	P8620
IV	5αP3α11β17α20β21	2630	3420	1021**	Calculated; L_R 5αP3α11β20β*** + ΔG_R§
V	5βP11β17α20β21(3)	2679	3428	1016**	Calculated; L_R 5βP11β20β(3)*** + ΔG_R§
VI	5βP3α11β17α20β21	2524	3402	981**	P8590; prepared 2 h RD P9550 and P7100; *cf.* Table I
VII	5αP11β17α20β21(3)	3076	3488	1035	Calculated; L_R 5αP11β20β(3)*** + ΔG_R§
VIII	Δ4P3β11β17α20β21	3182	3503	1020**	Prepared; 2 h RD Q2500; *cf.* Table I
IX	Δ5P3β11β17α20β21	3360	3526	1029	Prepared; 2 h RD Q5790; *cf.* Table I
X	5αP3β11β17α20β21	3472	3540	1034	Q4350; prepared 2 h RD P5200; *cf.* Table I
XI	Δ4P11β17α20β21(3)	3667	3564	1033	Q3790

* Average G_R-normal value = G_R P11β17α20β21 = 1032.
** G_R-odd steroid.
*** For L_R value, see Table XI of ref. 2.
§ ΔG_R = 478; *cf.* Table XVI.

TABLE XII

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP P11β17α20α21

Steroid					*Source(s)*
M	*Abbreviation*	t'_{NR}	L_R	G_R*	
I	5βP11β17α20α21	1279	3107	994**	Calculated; L_R 5βP11β20α*** + ΔG_R§
II	5αP11β17α20α21	1426	3154	1004	Calculated; L_R 5αP11β20α*** + ΔG_R§
III	5βP3β11β17α20α21	2523	3402	1000**	Calculated; L_R 5βP3β11β20α*** + ΔG_R§
IV	5αP3α11β17α20α21	2518	3401	1002**	Calculated; L_R 5αP3α11β20α*** + ΔG_R§
V	5βP11β17α20α21(3)	2588	3413	1000**	Calculated; L_R 5βP11β20α(3)*** + ΔG_R§
VI	5βP3α11β17α20α21	2433	3386	965**	SRC; prepared 2 h RD P9550 and P7100; *cf.* Table I
VII	5αP11β17α20α21(3)	2924	3466	1013	Calculated; L_R 5αP11β20α(3)*** + ΔG_R§
VIII	Δ4P3β11β17α20α21	3000	3477	994**	Prepared; 2 h RD Q2500; *cf.* Table I
IX	Δ5P3β11β17α20α21	3180	3502	1005	Prepared; 2 h RD Q5790; *cf.* Table I
X	5αP3β11β17α20α21	3295	3518	1012	Prepared; 2 h RD P5200; *cf.* Table I
XI	Δ4P11β17α20α21(3)	3467	3540	1009	Q3760

* Average G_R-normal value = G_R P11β17α20α21 = 1007.
** G_R-odd steroid.
*** For L_R value, see Table XII of ref. 2.
§ ΔG_R = 409; *cf.* Table XVI.

TABLE XIII

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

Steroid					*Source(s)*
M	*Abbreviation*	t'_{NR}	L_R	G_R**	
I	5βA(17)	140.5	2148	261	Prepared; *cf.* ref. 1
II	5αA(17)	154	2187	263	SRC
III	5βA3β(17)	276	2441	266	A3670
IV	5αA3α(17)	275	2439	264	A2420
V	5βA(3,17)	279	2445	261	A3270
VI	5βA3α(17)	288.5	2460	267	A3610
VII	5αA(3,17)	309	2489	261	A1630
VIII	Δ4A3β(17)	331	2520	263	Calculated; M_R Δ4A3β + G_R(17)**
IX	Δ5A3β(17)	337	2528	259	A8500
X	5αA3β(17)	348	2542	263	A2490
XI	Δ4A(3,17)	368	2566	261	A8090

* *Cf.* Table IX of ref. 1.
** Average G_R-normal value = G_R A(17) = 263.

TABLE XIV

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP A(11,17)*

Steroid					*Source(s)*
M	*Abbreviation*	t'_{NR}	L_R	G_R**	
I	5βA(11,17)	177	2248	361	Prepared*
II	5αA(11,17)	194	2287	363	Prepared*
III	5βA3β(11,17)	343	2535	361	Calculated; M_R 5βA3β* + G_R (11,17)**
IV	5αA3α(11,17)	342	2534	359	A2280
V	5βA(3,11,17)	336	2526	342***	A4010
VI	5βA3α(11,17)	348	2541	348***	A3460
VII	5αA(3,11,17)	379	2578	350***	Prepared*
VIII	Δ4A3β(11,17)	398	2599	342***	Calculated*
IX	Δ5A3β(11,17)	424	2627	358	SRC
X	5αA3β(11,17)	440	2643	364	Prepared*
XI	Δ4A(3,11,17)	426	2629	324***	Calculated*

* *Cf.* Table V of ref. 1.
** Average G_R-normal value = G_R A(11,17) = 361.
*** G_R-odd steroid.

TABLE XV

VALUES OF L_R AND G_R, AND SOURCES OF STEROIDS OF GROUP A11β(17)*

Steroid					*Source(s)*
M	*Abbreviation*	t'_{NR}	L_R	G_R**	
I	5βA11β(17)	224	2350	463***	Prepared*
II	5αA11β(17)	251	2399	475	Calculated*
III	5βA3β11β(17)	440	2643	468***	Calculated*
IV	5αA3α11β(17)	431	2634	459***	A1330
V	5βA11β(3,17)	441	2644	460***	Prepared*
VI	5βA3α11β(17)	442	2645	452***	A3120
VII	5αA11β(3,17)	503	2704	476	A2360
VIII	Δ4A3β11β(17)	523	2720	463***	Calculated*
IX	Δ5A3β11β(17)	558	2746	477	Calculated*
X	5αA3β11β(17)	571	2757	478	A1500
XI	Δ4A11β(3,17)	601	2779	474	A6630

* *Cf.* Table VI of ref. 1.
** Average G_R-normal value = G_R A11β(17) = 477.
*** G_R-odd steroid.

TABLE XVI

ΔG_R values*

Group (a)	*Group (b)***											
	A(11) (150)	*A(11,17) (361)*	*A17β(11) (507)*	*P(11) (156)*	*P(11,20) (370)*	*P20β(11) (511)*	*P20α(11) (523)*	*P17α20β(11) (688)*	*P20β21(11) (839)*			
P17α20β(11) (654)	730	519	374	498	283	141		654				
P17α20α(11) (688)							166					
P20β21(11) (839)	917	706	561	685	469	328						
P17α20β21(11) (969)	1046	835	690	814	598	457		315	130			
	A11β (222)	*A11β(17) (477)*	*A11β17β (568)*	*P11β (222)*	*P11β(20) (464)*	*P11β20β (555)*	*P11β20α (599)*	*P11β17α20β (723)*	*P11β20β21 (881)*	*P11β17α20α (757)*	*P11β20α21 (862)*	
P11β17α20β (723)	726	472	381	498	257	168						
P11β17α20α (757)							158					
P11β20β21 (881)	886	631	542	659	417	328						
P11β20α21 (862)							262					
P11β17α20β21 (1032)	1037	783	690	809	568	478		308	150			
P11β17α20α21 (1007)	1013	758	667	786	545	453	409			252	147	

* ΔG_R (a, b) = L_R (a) − L_R (b), *cf.* eqn. 13 (ref. 1), where L_R (a) and L_R (b) are L_R values of *M*-corresponding steroids in related groups (a) and (b). This table gives the average ΔG_R value for steroids of group (a), listed in column 1, and group (b), listed in rows. For example, the average ΔG_R value for groups P20β21(11) and P20β(11) is 328. These values closely approximate G_R (a) − G_R (b), where G_R (a) and G_R (b) are the G_R values of groups (a) and (b), respectively. The G_R values are given in parentheses beside or below the corresponding groups. When group (b) is of the androstane series, ΔG_R (a, b) = G_R (a) − G_R (b) + 226 (*cf.* eqn. 17, ref. 1).

** L_R values corresponding to groups A(11,17) and A11β(17) are listed in Tables XIV and XV, respectively. For L_R values corresponding to groups A(11), A11β, A17β(11) and A11β17β, see Tables III, IV, VII, and VIII of ref. 1, respectively. For L_R values corresponding to groups P(11), P11β, P(11,20), P11β(20), P20β(11), P20α(11), P11β20β, and P11β20α, see Tables V–XII of ref. 2, respectively.

TABLE XVII

MEAN DIFFERENCES, ΔG_R, OF L_R VALUES FOR *M*-CORRESPONDING 20α- AND 20β-STEROIDS

Group		ΔG_R*	*Source*
P20α	P20β	+28	Table XVIII of ref. 2
P20α(11)	P20β(11)	+13	
P11β20α	P11β20β	+49	
P17α20α	P17α20β	+24	Table XIV of ref. 4
P20α21	P20β21	−23	
P17α20α21	P17α20β21	−21	
P17α20α(11)	P17α20β(11)	+38	Calculated from present data; see Tables III–VI,
P11β17α20α	P11β17α20β	+38	VIII and IX, and XI and XII
P11β20α21	P11β20β21	−18	
P11β17α20α21	P11β17α20β21	−20	

* ΔG_R is expressed in L_R units. For specific *M*-configurations, the deviation from the mean ΔG_R value, ε (*cf.* Table XVIII of ref. 2) should be added to G_R. The only exception to this rule is the large ΔG_R, +55, for 5βP3α11β17α20α and 5βP3α11β17α20β (*cf.* Tables XI and XII).

separated TLC fractions in order of decreasing polarity, as shown by the corresponding R_b values of Δ4P-corticosteroids in brackets:

Δ4P11β17α21 (3,20), cortisol [0.142]
Δ4P17α21 (3,11,20), cortisone [0.300]
Δ4P11β21 (3,20), corticosterone [0.388]
Δ4P17α21 (3,20), cortexolone [0.468]
Δ4P21 (3,11,20), dehydrocorticosterone [0.660]
Δ4P21 (3,20), cortexone [0.832]

Further decisive structural information is gained by the application of appropriate discriminatory tests based on the use of reactions described in the present series. Such tests are very sensitive, often requiring less material than is needed to produce high-resolution mass spectra for positive identification and, therefore, requiring less extensive and time-consuming preparative effort. While this advantage is appreciable when dealing with the very low steroid levels found in animal blood and tissues, the operating costs of the method are also much lower[2]. Last, but not least, the method does not require samples of standard steroid for comparison because both the preliminary and the final identification can be made directly from predictable TLC and GLC properties. Hence, with this method, the current unavailability of many steroid standards no longer constitutes a serious obstacle to the systematic analysis of these compounds.

The simplicity of the method contrasts with the complexity of the present, extensive investigation on which it is based. This is evident, for example, from the essential GLC requirements for its effective application, namely: (1) a strict adherence to the type of non-polar column and conditions selected, including the use of two internal standards[1] and (2) standardization of the column, *i.e.*, determination of ΔG_R values from readily available steroid standards by rapid, systematic procedures as described in ref. 1.

TABLE XVIII

ERROR* ON L_R VALUES CALCULATED BY THE ΔG_R METHOD** FOR STEROIDS OF GROUPS*** P17α20β21(11), P11β17α20β21, AND P11β17α20α21

Steroid	*Error on L_R values*							
	A(11)	*A(11,17)*	*P(11)*	*P(11,20)*	*A17β(11)*	*P20β(11)*	*P17α20β(11)*	*P20β21(11)*
5βP3α17α20β21(11)	+26	+30	+30	+28	+15	+17	+16	− 4
Δ4P3β17α20β21(11)	0	− 2	+ 1	− 1	0	+ 1	0	+ 2
5αP3β17α20β21(11)	− 1	0	− 1	− 2	− 1	− 1	+ 2	0
	A11β	*A11β(17)*	*P11β*	*P11β(20)*	*A11β17β*	*P11β20β*	*P11β17α20β*	*P11β20β21*
5βP3α11β17α20β21	+27	+26	+29	+25	+ 2	+14	+ 2	− 5
Δ4P3β11β17α20β21	− 1	0	− 2	− 2	− 2	0	− 2	0
Δ5P3β11β17α20β21	+ 2	+ 3	+ 1	+ 1	+ 1	+ 2	+ 1	+ 2
5αP3β11β17α20β21	+ 1	0	+ 1	0	0	+ 2	− 1	+ 1
	A11β	*A11β(17)*	*P11β*	*P11β(20)*	*A11β17β*	*P11β20α*	*P11β17α20α*	*P11β20α21*
5βP3α11β17α20α21	+19	+17	+22	+18	− 5	+15	+17	−20
Δ4P3β11β17α20α21	+ 1	+ 1	+ 1	+ 1	+ 1.	0	+ 1	− 1
Δ5P3β11β17α20α21	+ 2	+ 2	+ 2	+ 2	+ 2	+ 1	+ 1	+ 1
5αP3β11β17α20α21	− 1	− 3	0	− 1	− 1	− 2	− 1	+ 1
Δ4P11β17α20α21(3)	− 2	− 3	− 3	− 2	− 3	− 1	0	0

* Expressed in L_R units.

** L_R values calculated as L_R (a) = L_R (b) + ΔG_R (eqn. 15 of ref. 1), where L_R (b) is the L_R value of the *M*-corresponding steroid in the related group indicated in the row, and ΔG_R is the appropriate value taken from Table XVI. L_R values of steroids of groups A(11,17) and A11β(17) are listed in Tables XIV and XV, respectively. L_R values of steroids of groups P17α20β(11), P11β17α20β, P11β17α20α, P20β21(11), P11β20β21, and P11β20α21 are listed in Tables III–IX, respectively. For L_R values of steroids of groups A(11), A11β, A17β(11), and A11β17β, see Tables III, IV, VII, and VIII of ref. 1, respectively. For L_R values of steroids of groups P(11), P11β, P(11,20), P11β(20), P20β(11), P11β20β, and P11β20α, see Tables V, VI, VII, VIII, IX, XI, and XII of ref. 2, respectively.

*** L_R values of steroids of these groups are listed in Tables X, XI, and XII, respectively.

By ensuring an excellent reproducibility and reliability of L_R data, the refined techniques which help to meet the first requirement also considerably reduce the amount of work required for effective operation. Once acquired, L_R and ΔG_Rvalues constitute a set of permanent, reliable constants characteristic of the system

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