

## CHROMATOGRAPHIC DATA OF PROGESTERONE ANALOGUES\*

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## INTRODUCTION

Paper chromatography as described by ZAFFARONI<sup>1</sup> is employed in many laboratories for the separation and characterization of steroids. Over the past two years we have collected considerable data on the chromatographic properties of progesterone and compounds of similar structure.

## MATERIALS AND METHODS

The developing systems employed formamide impregnated Whatman No. 2 paper as the stationary phase and Skellysolve B (boiling range 60–69°) and/or benzene as the mobile phase<sup>1</sup>. The chromatograms were run for 2–4 h at room temperature. The steroid spots were located by means of their absorption of ultraviolet light in a Haines-type scanner<sup>2</sup>. 4-Hydroxy-progesterone did not give a visible spot by this method and a ferric chloride–potassium ferricyanide reagent<sup>3</sup> was used to demonstrate this compound. All solvents were purified by standard methods<sup>4</sup>.

## RESULTS AND DISCUSSION

The results are tabulated in Tables I–IV. It should be noted that the mono-hydroxy analogues are recorded by  $R_F$  values whereas the  $R_P$  value is reported for each of the remaining compounds.  $R_P$  is defined as the distance traveled by the unknown steroid divided by the distance traveled by progesterone when the latter was run on the same chromatogram.

In all the compounds studied, with one exception, the presence of an alcoholic group increased the polarity (decreased the  $R_F$ ) with reference to progesterone. The congeners bearing primary or tertiary hydroxyl groups were, as a group, less polar than those with secondary hydroxyl groups. However, there are exceptions in each group. Thus, there is no progression of polarity with respect to the carbon atom to which the hydroxyl group is bonded.

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TABLE I  
*R<sub>F</sub>*'S OF MONO-HYDROXY CONGENERS OF PROGESTERONE

Substituent position	Substituent orientation*	<i>R<sub>F</sub></i> in solvent system**		
		S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>
2 $\alpha$ -	e	0.08	0.58	0.87
4-		0.28	0.60	
6 $\beta$ -	a		0.16	0.63
7 $\alpha$ -	a		0.08	0.41
9 $\alpha$ -	a		0.23	0.61
11 $\alpha$ -	e		0.14	0.38
11 $\beta$ -	a		0.29	0.57
12 $\alpha$ -	a		0.16	0.63
14 $\alpha$ -	a		0.23	0.59
15 $\alpha$ -	q		0.06	0.37
15 $\beta$ -	q		0.10	0.29
16 $\alpha$ -	q		0.06	0.30
17 $\alpha$ -	q	0.02	0.40	0.78
18-			0.34	0.64
19-				0.31
21-			0.33	0.83
Progesterone		0.3	0.9	1 (at front)

\* e = equatorial; a = axial; q = quasi.

\*\* Solvent systems: S<sub>1</sub> = Skellysolve B/formamide; S<sub>2</sub> = Skellysolve B-benzene (1:1)/formamide; S<sub>3</sub> = Benzene/formamide.

After studying a limited number of hydroxy-analogues of progesterone, NEHER<sup>5</sup> concluded that a hydroxyl group of equatorial orientation conferred more polarity to the molecule than did an axial group. In our data a true axial and equatorial pair was available only at the 11-carbon, and in this case NEHER's conclusion was substantiated. In general, however, there is no correlation between orientation and polarity when two different positions on the molecule are compared. Of interest is the extremely low polarity observed for 4-hydroxy-progesterone which may be explained by interaction between the hydroxyl group and the ketone function at carbon 3.

The substitution of a methyl group for a hydrogen atom on the progesterone molecule decreased the polarity of the molecule. Of the available pairs of congeners (2,6,7) the equatorial member exceeds the axial partner in polarity at carbon 2 whereas the axial methyl compound displayed greater polarity than the equatorial at carbons 6 and 7.

Many physical and chemical properties of halogen-containing compounds vary as the increasing size of the halogen atoms, *i.e.* fluorine, chlorine, bromine and iodine. The compounds tabulated in Table III are only a few of the theoretically possible halogen congeners of progesterone, and at no one position were all four halogen analogues available for study. With respect to polarity the following relationships were noted: 2 $\alpha$ -F > 2 $\alpha$ -I, 4-Cl = 4-Br, 6 $\alpha$ -F > 6 $\alpha$ -Cl, 6 $\beta$ -F  $\geq$  6 $\beta$ -Cl, 17 $\alpha$ -F = 17 $\alpha$ -Br. Thus, there is a suggested inverse relationship between the size of the substituent group and the polarity of the molecule bearing the group, but insufficient information is available. It is significant to note that the fluoro group does not confer polarity to the molecule in a degree comparable to the hydroxyl group.

TABLE II  
*R<sub>P</sub>*'S OF MONO-METHYL CONGENERS OF PROGESTERONE\*

Substituent position	Substituent orientation	<i>R<sub>P</sub></i> <i>S</i> <sub>1</sub>
2 $\alpha$ -	e	1.64
2 $\beta$ -	a	1.78
4-		1.42
6 $\alpha$ -	e	1.31
6 $\beta$ -	a	1.18
7 $\alpha$ -	a	1.04
7 $\beta$ -	e	1.22
16 $\alpha$ -	q	1.33
16 $\beta$ -	q	1.03
17 $\alpha$ -	q	1.43

\* For abbreviations see Table I.

TABLE III  
*R<sub>P</sub>*'S OF MONO-HALO CONGENERS OF PROGESTERONE\*

Substituent position	Substituent orientation	<i>R<sub>P</sub></i>	
		<i>S</i> <sub>1</sub>	<i>S</i> <sub>2</sub>
2 $\alpha$ -Fluoro	e	0.17	0.86
2 $\alpha$ -Iodo	e	1.25	1.05
4-Chloro		0.2	0.7
4-Bromo		0.18	0.76
6 $\alpha$ -Fluoro	e	0.21	0.53
6 $\beta$ -Fluoro	a	0.63	0.56
6 $\alpha$ -Chloro	e	0.77	0.68
6 $\beta$ -Chloro	a	0.63	0.61
16 $\alpha$ -Chloro	q	0.53	
17 $\alpha$ -Fluoro	q	1.65	1
17 $\alpha$ -Bromo	q	1.65	1

\* For abbreviations see Table I.

TABLE IV  
*R<sub>P</sub>*'S OF DEHYDRO CONGENERS\*

Double bond position	<i>R<sub>P</sub></i>	
	<i>S</i> <sub>1</sub>	<i>S</i> <sub>2</sub>
1	0.38	0.97
6	0.71	1.07
8	0.75	0.85
9 (II)	0.96	0.96
11	0.81	0.92
14	0.75	0.8
16	0.81	0.63

\* For abbreviations see Table I.

Compounds differing from progesterone only by their containing an additional double bond were generally more polar than the reference steroid. It is of interest that 6-dehydro-progesterone was less polar than progesterone even though a double bond at 6 extends the 4-3-ketone conjugation.

#### SUMMARY

Several substituted steroids having the same nuclear structure as progesterone have been studied in Zaffaroni type chromatographic systems. In general it was found that hydroxyl groups increase the polarity of the resultant molecule as do additional double bonds and halogen substituents. Methyl congeners showed decreased polarity as compared with progesterone.

#### REFERENCES

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