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Note

Gas–liquid chromatography of keto- and hydroxyandrostanes

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Earlier work on the gas–liquid chromatography (GLC) of steroids[1] showed that the ratio of their retention volumes on polar and non-polar stationary phases was characteristic of the nature of the substituent group. Moreover it was found that the position of the group on the ring and its equatorial or axial configuration influenced the retention ratio assigned to the substituent group.

The present study is concerned with a single steroid nucleus, androstane. The GLC properties of the hydroxyandrostanes and the corresponding ketones have been investigated. These two series of compounds were chosen for the study because the two substituents are capable of specific interaction with polar solvents, but only the hydroxy compounds allow axial and equatorial substitution. Thus the variation in retention ratio in the ketone series would be due only to the position on the androstane rings of the substituent, while the configurational variation should also be apparent in the results for the hydroxy series. It was, therefore, anticipated that the value of the retention ratio would give a good indication, not only of the extent of interaction with the solvent in GLC, but also of the molecular geometry of substituted androstanes.

EXPERIMENTAL

A Pye 104 chromatograph fitted with dual columns and flame ionisation detectors was used. Inlet pressures to the two columns were monitored using high precision bourdon type pressure gauges; carrier gas flow-rates (nitrogen) were measured using soap-bubble flow-meters. Initial experiments showed that retention volumes were independent of carrier gas flow-rate up to 80 ml/min, and present work utilised a flow-rate of 30 ml/min. Under such conditions the column efficiencies were sufficiently high (greater than 2000 theoretical plates) to enable complete separation under the conditions used, of all pairs of components studied. The use of dual columns mounted in the same oven ensured comparison between the polar and non-polar solvents at identical temperatures.

Glass columns nominally 5 ft. × 0.25 in. O.D. were packed with 1% SE-30 or neopentyl glycol sebacate (NPGS) on HMDS-treated Chromosorb G (100–120 mesh, ASTM), a system designed to limit adsorption on the free surface of the columns.

Packings were prepared by coating the stationary phase from solution and evaporating the volatile solvent under a stream of nitrogen.

Samples were presented as 0.03% (w/v) solutions in carbon tetrachloride, 0.02% androstane being added as an internal standard. The sample size up to 5 μl had no effect on the retention volume and a sample size of 1 μl was used in the experiments.

Measurement of heats of solution were carried out at 217° and 190° for the hydroxyandrostanes, and 197° and 217° for the ketoandrostanes, conditions under which the longest retention times were about 70 min.

Oven temperatures were measured with a single mercury-in-glass thermometer with a standard extent of immersion, which was subsequently calibrated against an accurately standardized total immersion thermometer. No temperature change greater than 0.05° was noted even over the longest runs.

Retention volumes quoted are the mean of three independent determinations: the maximum deviation from the mean of the relative retention volumes is $\pm$ 0.25%. The dead volume of each column was determined by injection of methane, which, at the temperatures involved, may be treated as totally insoluble in the stationary phase. Retention times were measured as the distances from the methane peak to the peak maxima. Relative retention data are expressed as the ratio of chart distances. The recorder motor speed showed a deviation of $<$ 0.05% over any 2-h period.

TABLE I

RELATIVE RETENTION VOLUMES (V_{OH}) AT 190° FOR HYDROXYANDROSTANES

These figures and those of Tables II and III correspond to the group retention factors of Clayton[4].

Compound (hydroxy position)	V_{OH}*		*Ratio* $V_{OH(b)}/V_{OH(a)}$
	SE-30 (a)	*NPGS (b)*	
2α (e)	2.23	5.63	2.53
2β (a)	2.13	4.95	2.32
3α (a)	2.22	5.22	2.35
3β (e)	2.29	5.69	2.49
4α (e)	2.20	5.23	2.37
4β (a)	2.09	4.57	2.19
6β (a)	1.99	4.41	2.22
7α (a)	1.99	4.09	2.06
7β (e)	1.99	4.37	2.19
11α (e)	1.89	4.05	2.14
12α (a)	2.18	4.93	2.26
12β (e)	2.16	5.30	2.45
15α	1.97	4.73	2.40
15β	1.97	4.36	2.21
16α	2.23	5.46	2.45
16β	2.25	5.39	2.40
17α	2.25	5.21	2.32
17β	2.28	5.56	2.43

* 5α-Androstane = 1.

RESULTS

The relative retention data for the hydroxyandrostanes are shown in Tables I and II. The axially and equatorially substituted compounds are indicated by (a) and (e), respectively. Table III shows the retention data for the ketoandrostanes.

TABLE II

RELATIVE RETENTION VOLUMES (V_{OH}) AT 217° FOR HYDROXYANDROSTANES

Compound (hydroxy position)	V_{OH}*		*Ratio* $V_{OH(2)}/V_{OH(1)}$
	SE-30 (1)	*NPGS (2)*	
2α (e)	2.00	4.45	2.23
2β (a)	1.95	3.98	2.04
3α (a)	1.96	4.15	2.12
3β (e)	2.02	4.60	2.28
4α (e)	1.99	4.18	2.11
4β (a)	1.89	3.72	1.97
6β (a)	1.85	3.71	2.01
7α (a)	1.77	3.41	1.93
7β (e)	1.81	3.54	1.96
11α (e)	1.76	3.44	1.96
12α (a)	1.99	4.10	2.06
12β (e)	1.99	4.41	2.22
15α	1.80	4.01	2.23
15β	1.80	3.70	2.06
16α	1.97	4.31	2.18
16β	1.99	4.25	2.13
17α	2.00	4.21	2.11
17β	2.07	4.40	2.13

* 5α-Androstane = 1.

TABLE III

RELATIVE RETENTION VOLUMES (V_{CO}*) FOR KETOANDROSTANES

Compound (keto position)	V_{CO} *at 197°*		*Ratio (197°)* $V_{CO(2)}/V_{CO(1)}$	V_{CO} *at 217°*		*Ratio (217°)* $V_{CO(2)}/V_{CO(1)}$
	SE-30 (1)	*NPGS (2)*		*SE-30 (1)*	*NPGS (2)*	
1	1.80	3.22	1.79	1.67	3.00	1.80
2	2.17	4.79	2.20	2.00	4.30	2.14
3	2.34	5.49	2.35	2.17	4.95	2.28
4	2.10	4.41	2.10	1.99	3.93	1.97
6	2.05	4.31	2.10	1.95	3.87	1.98
7	1.97	3.85	1.95	1.85	3.54	1.92
11	1.53	2.59	1.69	1.49	2.44	1.64
12	2.18	4.53	2.08	2.06	4.09	1.99
15	1.67	3 04	1.82	1.60	2.76	1.72
16	2.11	4.71	2.23	2.00	4.23	2.12
17	2.10	4.37	2.08	1.99	3.92	1.97

* 5α-Androstane = 1.

TABLE IV

ENTHALPY DATA FOR HYDROXYANDROSTANES

Compound (hydroxy position)	$-(\Delta H^0_{OH}-\Delta H^0_A)$ *(J/mole)*		$-\Delta(\Delta H^0_{OH}-\Delta H^0_A)$ *(J/mole) (interaction enthalpy)*
	SE-30	*NPGS*	
2α (e)	7700	16 000	8200
2β (a)	6300	15 000	8800
3α (a)	8300	16 000	7700
3β (e)	8600	15 000	6400
4α (e)	6800	15 600	8800
4β (a)	9000	14 700	5700
6β (a)	5100	12 100	7000
7α (a)	8100	12 500	4400
7β (e)	6800	14 700	7900
11α (e)	4800	11 400	6600
12α (a)	6300	12 800	6500
12β (e)	5700	12 800	7100
15α	6300	11 600	5300
15β	6400	11 400	5200
16α	8400	16 300	7800
16β	8300	16 600	8300
17α	7900	15 100	7100
17β	7500	16 400	8800

Tables IV and V show some heat of solution data derived by a Van 't Hoff method. Application of the solution isochore to the relative retention volumes yields the equation:

$$\frac{R \log_e \frac{V_{OH(2)}}{V_{OH(1)}}}{\left(\frac{1}{T_1}-\frac{1}{T_2}\right)} = \Delta H^0_{OH} - \Delta H^0_A$$

in which R is the gas constant, ΔH^0_{OH} is the enthalpy of solution of the hydroxyandrostane vapour and ΔH^0_A is the enthalpy of solution of the androstane. The subscripts refer to the two different column conditions. The values of $\Delta H^0_{OH}-\Delta H^0_A$ in Tables IV and V were calculated using this equation.

If SE-30 is considered to be non-polar then the values of $-(\Delta H^0_{OH}-\Delta H^0_A)$ evaluated from data obtained on an SE-30 column are a measure of the change in vaporisation enthalpy induced in the androstane by the introduction of a hydroxyl group. The value of $-(\Delta H^0_{OH}-\Delta H^0_A)$ obtained from retention data on the NPGS column also includes the contribution of the hydroxyl group to the enthalpy of mixing. Thus the difference between these values of $-(\Delta H^0_{OH}-\Delta H^0_A)$ is a measure of the interaction enthalpy between the hydroxyl group and the polar solvent. Values of this difference $[-\Delta(\Delta H^0_{OH}-\Delta H^0_A)]$ are tabulated as interaction enthalpies in Tables IV and V.

Table VI shows some relative retention data for the trimethyl silyl (TMS) ethers of the hydroxyandrostanes.

TABLE V

ENTHALPY DATA FOR KETOANDROSTANES

Keto position	$-(\Delta H^0_{OH}-\Delta H^0_A)$ *(J/mole)*		$-\Delta(\Delta H^0_{OH}-\Delta H^0_A)$ *(J/mole) (interaction enthalpy)*
	SE-30	*NPGS*	
1	7400	6600	-800
2	7700	13200	5500
3	7000	9900	2900
4	5100	10900	5800
6	4500	10500	6000
7	6000	8100	2100
11	2900	6100	3200
12	5500	9600	4100
15	4000	9000	5000
16	5400	10200	4800
17	5400	10400	5000

TABLE VI

RELATIVE RETENTION TIMES,*,** (V_{TMS}) OF TMS ETHERS OF HYDROXYANDROSTANES ON NPGS AT 217 °C

Compound (hydroxy position)	V_{TMS}
2α (e)	1.99
2β (a)	1.70
3α (a)	1.60
3β (e)	2.37
4α (e)	2.06
4β (a)	1.78
6α (e)	1.56
6β (a)	1.22
7β (e)	1.64
16α	1.97
16β	2.03
17α	2.08
17β	2.10

* Sequence: equatorial, $3\beta > 4\alpha > 2\alpha > 7\beta$; axial, $4\beta > 2\beta > 3\alpha$; 5-ring, $17\beta > 17\alpha > 16\beta > 16\alpha$.
** 5α-Androstane = 1.

DISCUSSION

Of the eighteen hydroxyandrostanes examined, twelve are substituted in rings A, B, and C. For these the results in Tables I and II show that, on the more selective NPGS and at the lower temperature, the less hindered equatorial isomers have a significantly greater retention volume than the axial epimers. The difference is most marked in those cases such as C-2, C-4, and C-6 in which a β-axial group is present, a configuration more subject to steric hindrance than for example the α-axial compounds C-3, C-7, and C-12. On SE-30 such differences are less marked and with the hindered 7-position disappear altogether. These results confirm and extend previous findings[1-3].

For rings A, B, and C the retention volume sequences are: equatorial, $3\beta > 2\alpha \equiv 12\beta > 4\alpha > 7\beta > 11\alpha$; axial, $3\alpha > 12\alpha \equiv 2\beta > 4\beta > 6\beta > 7\alpha$. In the axial series, the compounds substituted on the less hindered α face of the androstane nucleus, give the longer retention times.

Ring D substituents yield the order, $17\beta > 16\alpha > 16\beta > 17\alpha > 15\alpha > 15\beta$: despite the juxtaposition of the 17β-hydroxyl to the C-18 methyl group, substitution in this position surprisingly shows the longest retention time.

The ketoandrostanes on the other hand show the same general pattern on both stationary phases and at both temperatures examined, *e.g.* for NPGS at 217°, $3 > 2 > 16 > 12 > 4 \equiv 6 > 1 > 15 > 11$ whilst on SE-30 the only difference is to displace the 12-ketoandrostane to a position between 3 and 2.

The order of elution of the TMS ethers of the hydroxyandrostanes (Table VI) follows more closely that for the free hydroxyandrostanes when the hydroxyl is in an equatorial position. This suggests that the equatorial hydroxyandrostanes adopt an edge on interaction with the polymer molecules.

The values of the enthalpy of interaction for the hydroxyandrostanes ranging from 4500 J/mole to 8800 J/mole suggest a rather weak hydrogen bonding and the differences between hydroxy- and ketoandrostanes (*ca.* 2500 J/mole) are consistent with such an interaction.

The enthalpy of interaction when the hydroxyl group is in the 17α-position is lower (7130 J/mole) than when it is in the 17β (8842 J/mole), 16α (7833 J/mole), or 16β position (8284 J/mole). This is surprising in view of the known hindrance between the C-18 methyl and a 17β-substituent; this is a further reflection of the anomalous retention time referred to above.

If the enthalpy of interaction for compounds with axial and equatorial substitution at any one position are compared, it can be seen that the value is slightly larger in the case of equatorial substitution (Table IV). Such interaction enthalpy differences are insufficient, however, to explain the quite large difference in retention ratio between equatorial and axial substituents and would suggest that the entropies of mixing between equatorially substituted androstanes and the solvent are substantially higher than those for axially substituted compounds. Consequently the androstanes with equatorial groups must be presumed to exhibit freer rotation when interacting with the polymeric solvent molecules than do the axially substituted androstanes.

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