## Retention behavior of steroids in gas chromatography with a series of combination columns

The present paper shows that a series of combination columns might be used as means for obtaining further information regarding the structure of steroidal compounds. Steroids predominantly ketonic showed a negative slope, while hydroxyl type compounds presented a positive slope when retention times were plotted against substrate concentration. Equal numbers of ketones and hydroxyls in a molecule resulted in little or zero slope.

*Experimental*

A Glowall Model A-110 with a 1 cm argon ionizing detector, containing radium, was used. Glass coil columns, 6 ft. × 4 mm I.D., were packed by the vacuum technique with Gas Chrom Q (Applied Science) coated with the stated concentration of substrate. The coating procedure involved dissolving the calculated amounts of both substrates in methylene chloride and evaporating the solvent after addition of the support. All the columns were conditioned at 240° for 18 h or more with argon flowing through at 10 p.s.i. inlet pressure. Flash temperature 260°, column and detector temperature 240°, with an inlet pressure of 30 p.s.i. are the operating conditions.

All steroids were used as obtained from the supplier. In each instance the amount of impurity was negligible for the present purpose.

Samples were made up as 0.5 $\mu$g/$\mu$l solution in *tert.*-butyl alcohol and 2–4 $\mu$g were injected using a 10 $\mu$l Hamilton syringe.

Relative retention times with respect to estrone as unity were calculated by measuring distance of maximum peak height from the initial pressure or "air" peak.

The retention times were plotted against percentage concentration of substrate in the combination packing used in the columns.

The pair QF-1 (fluorosilicone polymer, Dow Corning) and L-45 (methyl silicone, General Electric) were used since these in combination have given the most satisfactory results for separation of many steroids. They represent a selective and non-selective substrate respectively. In addition as previously reported[1], this type of combination can result in greater response or recovery for certain compounds. The packings were made up so that the total substrate concentration was 5 % of support; *viz.* 3.5 % of QF-1 and 1.5 % of L-45 or either one 5 % alone.

*Results*

Table I gives the retention times of 16 representative steroids relative to estrone as unity. These retention times are plotted in a nomographic manner in Fig. 1. Both estrone and androsterone, each having a single ketonic and hydroxylic function, showed zero slopes in the curve presented by their retention times. Progesterone, a diketone, had a negative slope while the dihydroxyl compound estradiol-17$\beta$ showed a positive slope. Compounds containing predominantly ketones had negative slopes, while those with more hydroxyls showed positive slopes. As in the case of cholesterol, molecular weight has an influence on the retention times while retaining the positive slope due to the hydroxyl function.

Preliminary attempts were made to apply regression lines to the data. If this could be done it would be possible to apply mathematical methods to predict the

TABLE I

RETENTION BEHAVIOR OF STEROIDS IN A SERIES OF COMBINATION COLUMNS RELATIVE TO ESTRONE (10 MIN) AS 100

| *Compound* | %QF-1[a] %L-45 | *Relative retention time* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5% 0 | | 3.75% 1.25% | | 2.5% 2.5% | | 1.25% 3.75% | | 0 5% | |
| | | 1[b] | 2[c] | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Cholesterol | | 105 | 108 | 170 | 163 | 235 | 240 | 300 | 301 | 365 | 364 |
| 2α-Hydroxyestriol | | 252 | 253 | 287 | — | 322 | 322 | 357 | — | 392 | 370 |
| 3α,20α-Dihydroxypregnane | | 78 | 80 | 91 | 90 | 104 | 105 | 117 | 118 | 130 | 131 |
| Estradiol-17β | | 65 | 67 | 75 | 75 | 86 | 85 | 95 | 95 | 105 | 105 |
| Estriol | | 144 | 143 | 153 | 153 | 162 | 163 | 171 | 171 | 180 | 181 |
| Androst-5-ene-3β,17α-diol | | 52 | 53 | 60 | 60 | 68 | 68 | 76 | 76 | 84 | 86 |
| Pregnanolone | | 106 | 106 | 110 | 111 | 113 | 114 | 117 | 117 | 120 | 121 |
| Androsterone | | 82 | 82 | 82 | 82 | 82 | 82 | 82 | 82 | 82 | 83 |
| Estrone | | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 17β-Hydroxyandrostan-3-one | | 106 | 106 | 103 | — | 100 | 100 | 97 | 98 | 94 | 95 |
| Testosterone | | 160 | 159 | 148 | 148 | 136 | 136 | 124 | 124 | 112 | 114 |
| Androstane-3,17-dione | | 180 | 179 | 158 | 161 | 136 | 136 | 114 | 111 | 92 | 92 |
| Androst-4-ene-3,17-dione | | 258 | 259 | 220 | 226 | 182 | 185 | 144 | 143 | 106 | 109 |
| Progesterone | | 332 | 332 | 292 | 293 | 252 | 256 | 212 | 211 | 172 | 173 |
| 17α-Hydroxyprogesterone | | 454 | 454 | 400 | — | 346 | 346 | 292 | — | 238 | 238 |
| Androst-4-ene-3,11,17-trione | | 402 | 406 | 332 | 341 | 262 | 271 | 192 | 193 | 122 | 127 |

[a] The columns used are not more than three days old. Retention times will change gradually with age.
[b] Calculated data.
[c] Experimental data, each figure represents the average of three determinations.

structure of a compound. Coefficients of determination for linear curves fitted to the 16 compounds were of the order 94–97 %. This was not considered sufficient for accurate prediction of molecular structures.

It has been shown by others that equatorial configurations in the various positions of the steroid molecule influence retention times. We are now in process of enlarging the series in attempt to improve the coefficient of determination.

Since the relationship between retention times and concentration of substrate was linear, a mathematical expression was possible:

$$\frac{P_1R_1 + P_2R_2}{P_1 + P_2} = R_3$$

The percentage of a second substrate ($P_2$) required to give a separation ($R_3$) when the amount of the other substrate ($P_1$) is known can be calculated. The retention times of $R_1$ and $R_2$ of the individual substances with the substrates individually must be known. In the experiments so far performed substrates combinations over 15 % were not satisfactory. This type of data has been adapted for computer use by PORTER *et al.*[2].

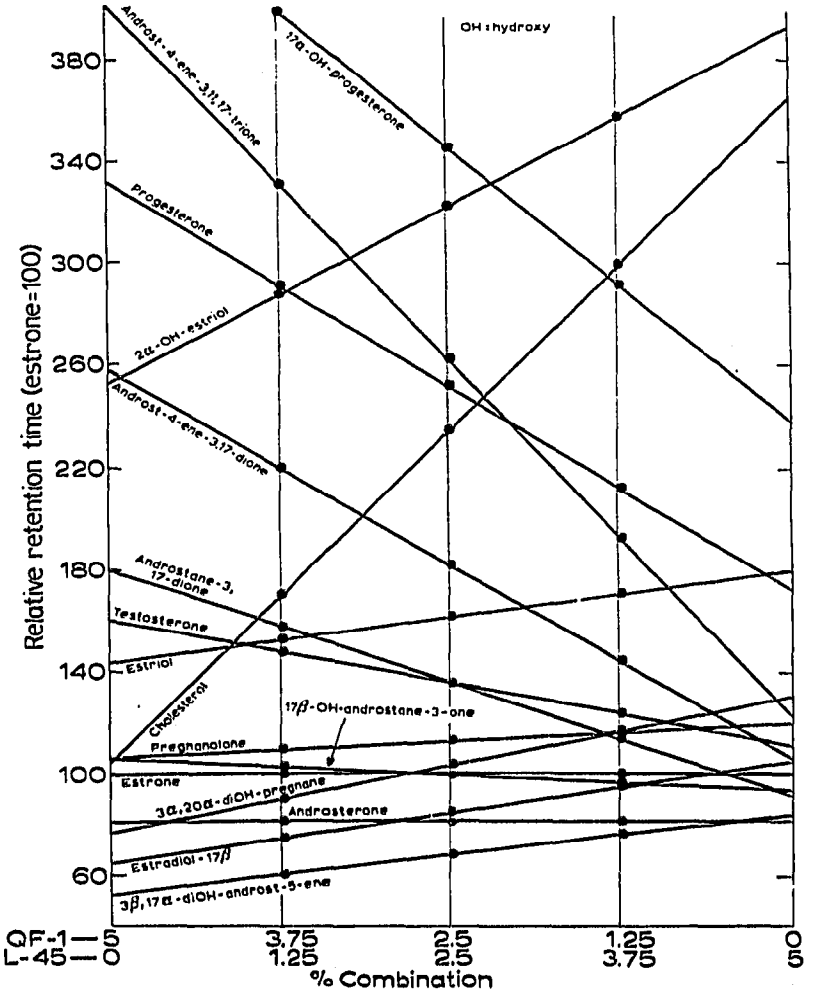


Fig. 1. Retention behavior of steroids on combination columns plotted from calculated data in Table I. OH = hydroxy.

*Discussion*

A number of reports have described the use of the steroid number concept[3–5]. Retention factors have been determined for a number of functions of the steroid molecule[6,7]. The KOVATS retention index system has been widely used as an aid to structural investigations[8]. KNIGHTS AND THOMAS[9] used the log of retention values which were additive for structural determination. HILDEBRAND AND REILLEY[10] showed how mathematical formulae may be used to predict the retention times of various compounds on combination columns.

There appears to be a correlation between the nature of the functional groups within the molecule and the slopes of the curves as obtained in the present work. It may be that determination of structure might be facilitated if a larger series including geometrical considerations were obtained and a mathematical expression derived.

By use of varying substrate proportions in the combination, if the relative retention times of a number of compounds are known, it is possible to calculate a combination to give the maximum resolution of a mixture. With a wide variety of substances present in biological samples this tool can be of advantage.

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1 J. C. TOUCHSTONE, A. NIKOLSKI AND T. MURAWEC, *Steroids*, 5 (1965) 423.
2 R. S. PORTER, R. L. HINKINS, L. TORNHEIM AND J. F. JOHNSON, *Anal. Chem.*, 36 (1964) 260.
3 R. B. CLAYTON, *Nature*, 192 (1961) 524.
4 E. O. A. HAAHTI, W. J. A. VANDENHEUVEL AND E. C. HORNING, *Anal. Biochem.*, 2 (1961) 182.
5 S. R. LIPSKY AND R. A. LANDOWNE, *Anal. Biochem.*, 3 (1961) 818.
6 W. J. A. VANDENHEUVEL, B. G. CREECH AND E. C. HORNING, *Anal. Biochem.*, 4 (1962) 191.
7 R. B. CLAYTON, *Biochemistry*, 1 (1961) 357.
8 E. Z. KOVATS, *Z. Anal. Chem.*, 181 (1961) 357.
9 B. A. KNIGHTS AND G. H. THOMAS, *Anal. Chem.*, 34 (1962) 1046.
10 G. P. HILDEBRAND AND C. M. REILLEY, *Anal. Chem.*, 36 (1964) 47.

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