R_M values in adsorption chromatography

The concept of R_M values developed by BATE-SMITH AND WESTALL[1] has been successfully applied to the analysis of steroids chromatographed in liquid–liquid[2] and gas–liquid[3] partition systems. LEDERER[4] has suggested that this method of analysis might usefully be extended to adsorption systems, using data easily obtained from thin-layer chromatography. This note is intended to show that, although general rules can be formulated for adsorption systems, the precise determination of ΔR_{M0} and ΔR_{Mr} values (as defined by BUSH[2]) is not as simple as in partition systems. Irregularities in relative elution properties have been noted and it is suggested[5] that these may be attributed to the fact that substances adsorbed onto rigid surfaces can possess different conformations from those which they have in solution. The following results also illustrate these points.

Steroids were chromatographed on Stahl chromatoplates[6] using as adsorbents Silica Gel G and Alumina G (E. Merck & Co., Darmstadt). For the purposes of comparison, the same steroids were chromatographed in a gas–liquid system using as stationary phase QF-1, a fluorinated alkyl silicone polymer[7]. Steroids on chromatoplates were detected as coloured spots after spraying with concentrated sulphuric acid in ethanol and heating at 110° for 10 minutes.

Table I shows that, in general, saturated ketones are eluted ahead of α,β-unsaturated ketones which are eluted ahead of alcohols on Silica Gel G when chloroform is the eluant.

Table II shows the ΔR_{Mr} values for the conversion of a 5α-3-ketone to a 5β-3-

TABLE I

R_F VALUES ON SILICA GEL G WITH CHLOROFORM AS ELUANT

Steroid	R_F*
5α-Pregnane-3,20-dione	0.86
5β-Pregnane-3,20-dione	0.85
5α-Androstane-3,17-dione	0.79
5β-Androstane-3,17-dione	0.65
Pregn-4-ene-3,20-dione	0.76
19-Norpregn-4-ene-3,20-dione	0.60
Androst-4-ene-3,17-dione	0.52
3α-Hydroxy-5β-pregnan-20-one	0.35
3β-Hydroxypregn-5-en-20-one	0.37
17β-Hydroxy-5β-androstan-3-one	0.36
3α-Hydroxy-5α-androstan-17-one	0.33
3β-Hydroxyandrost-5-en-17-one	0.35
20β-Hydroxypregn-4-en-3-one	0.25
17β-Hydroxyandrost-4-en-3-one	0.21
17β-Hydroxy-19-norandrost-4-en-3-one	0.21
5β-Pregnane-3α,20α-diol	0.10
Pregn-5-ene-3β,20β-diol	0.18
5β-Pregnane-3α,20β-diol	0.13
5α-Androstane-3β,17β-diol	0.14

* All steroids were chromatographed on a single plate.

TABLE II

COMPARISON OF ΔR_{Mr} (3-KETONE (5α) → 3-KETONE (5β)) VALUES IN ADSORPTION AND GAS–LIQUID PARTITION SYSTEMS

Compound	ΔR_{Mr} (3-ketone (5α) → 3-ketone (5β))		
	Adsorption systems		Gas-liquid partition system
	Alumina G	Silica Gel G***	6% QF-1 (250°)
Cholestan-3-one	— 0.26*	— 0.07	— 0.04
Pregnane-3,20-dione	+ 0.03*	+ 0.05	— 0.04
Androstane-3,17-dione	+ 0.40**	+ 0.14	— 0.04
17β-Hydroxyandrostan-3-one	+ 0.12**	+ 0.04	— 0.04

* Eluant: chloroform–toluene(1:3).
** Eluant: chloroform–benzene(1:1).
*** Eluant: benzene–chloroform (1:3).

ketone for two adsorption systems and the gas–liquid partition system. It can be seen that in the partition system the 5β-3-ketone is always eluted ahead of its 5α-isomer with a consistent ΔR_{Mr} value of — 0.04. No such relationship is found in the adsorption systems.

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1 E. C. BATE-SMITH AND R. G. WESTALL, *Biochim. Biophys. Acta*, 4 (1950) 427.
2 I. E. BUSH, *The Chromatography of Steroids*, Pergamon Press, Oxford, 1961.
3 B. A. KNIGHTS AND G. H. THOMAS, *Nature*, 194 (1962) 833.
4 M. LEDERER, *J. Chromatog.*, 9 (1962) 277.
5 R. V. BROOKS, W. KLYNE AND E. MILLER, *Biochem. J.*, 54 (1953) 212.
6 E. STAHL, *Chemiker Ztg.*, 82 (1958) 323.
7 W. J. A. VANDENHEUVEL, E. O. A. HAAHTI AND E. C. HORNING, *J. Am. Chem. Soc.*, 83 (1961) 1513.

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Some pitfalls in studies related to gas chromatography

This report discusses certain pitfalls in studies on natural products involving gas chromatography.

Qualitative and semiquantitative investigations on aroma-bearing constituents frequently involve procedures in which the constituents are ultimately obtained in an ether solution at very low concentrations, *e.g.*, 1 l of solution containing 0.01% solute. The ether solution is then evaporated to a small volume (*e.g.*, 1 ml or less)