PAPER CHROMATOGRAPHIC CHARACTERISTICS OF SUBSTITUTED DIPHENYLETHENES

I. *p,p'*-SUBSTITUTION IN THE PHENYL RINGS

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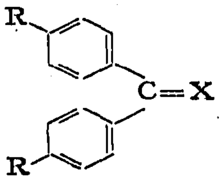
In the investigation of biological effects of diphenylethenes with the general formula



where X is alkylidene or cycloalkylidene, it was found desirable to compare the distribution characteristics of the compounds between a polar and a lipid phase. Owing to the large number of substances, paper chromatography was considered the most suitable method for the purpose in view.

In chromatography of homologous series of substances it is convenient to use R_M, instead of R_F; according to theory the former is a linear function of the number of similar substituents in a molecule. The R_M function was introduced by BATE-SMITH AND WESTALL[1], who in their investigation of phenols and flavones defined R_M as $\log(1/R_F - 1)$. They found a largely linear correlation between R_M and the number of hydroxyl and carboxyl groups. REICHL[2] modified the formula to $R_M = \log R_F/(1 - R_F)$. This modified formula is easier to handle. Any increase in the value of R_F is accompanied by a corresponding increase of R_M (Fig. 1). REICHL determined a fundamental constant and a group constant for all his solvents and found that the R_M value for a substance can be calculated by addition of these constants, this sum giving the R_M value. Among recent investigations of the influence of substituents on the chromatographic characteristics of the compounds studied, that of BARK AND GRAHAM[3], who studied phenoxyacetic acids, deserves mention as does that of KABASAKALIAN AND BASCH[4], who studied the steroids of the pregnane series with the aid of so-called Zaffaroni systems. For the dimethyl sulphoxide–water/isopropyl ether system, SOCZEWINSKI[5] found a linear correlation between R_M and per cent by volume of water in the stationary phase. He assumed that this correlation was generally valid and that thus similar relationships can also be expected for reversed phase chromatography, an assumption confirmed in the present investigation.

An advantage of the R_M value is that it is a simple function of the temperature and the relative volumes of the solvent phases. Using a standard substance for the chromatograms, any deviations from normal can be readily detected and corrected for in the formula.

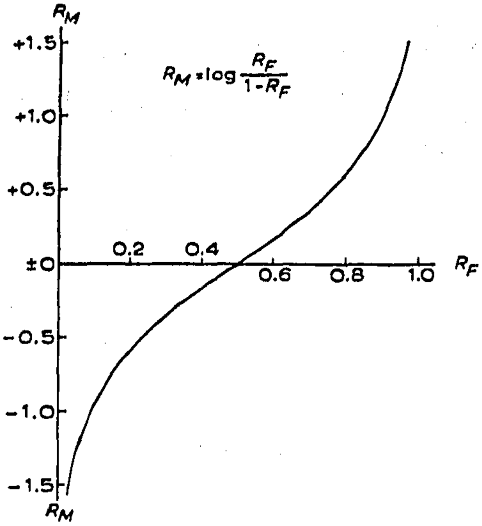


Fig. 1. The R_M function according to REICHL.

MATERIAL AND METHODS

The compounds studied were the substituted diphenylethenes described by MIQUEL *et al.*[6]. Substances that gave more than one spot on a chromatogram or showed other signs of contamination were recrystallized in some suitable solvent.

As reference substance, which was applied to every sheet of chromatographic paper, we used 1,1-bis-(*p*-acetoxyphenyl)-2-ethylbutene-1, recrystallized from 70 % ethanol and diisobutyl ketone to constant melting point. The values for the other spots were corrected with reference to the R_M value of the standard substance. The mean value for the R_F of the standard was calculated on the basis of 125 chromatograms. Six chromatograms in which the R_F of the standard substance differed by more than ± 0.03 from the mean value were excluded.

The chromatographic paper, Whatman No. 1, 33.5 by 31.5 cm, was impregnated by dipping it into a solution of 5 % by volume of paraffin oil in cyclohexane. Excess solution was removed by blotting the sheet between Whatman No. 3 MM papers, after which the sheet was allowed to dry in the air. Storage of the impregnated paper for a long period up to 6 months appeared to have no effect on the R_F values. The starting line was marked 4 cm from the short edge of the paper, and 10 μl of a solution of each substance (0.5 mg/ml ethanol) was deposited on the paper with the aid of a micropipette. The spots were placed 4 cm apart. The chromatogram was suspended vertically and developed by the ascending technique in "Shandon 13 inch Universal Sheet Chromatanks", containing 400 ml of the various mixtures of methanol and water. Five hours at 22–24° proved to be a suitable running time.

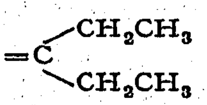
With the amount used, 5 γ substance per spot, well defined spots were obtained; larger amounts gave a certain degree of tailing, especially in the system with the highest water content.

After 5 hours the paper was removed from the tank, air dried, and examined in ultraviolet light (Hanovia Chromatolite, 253.7 mμ).

Most of the compounds studied have an absorption maximum between 240 and 260 mμ, so that the detection method is fairly sensitive, it being possible to recognize substances in amounts of 0.5 γ/cm^2 without difficulty. Substances with one or two phenolic hydroxyls could be demonstrated with about the same sensitivity with Folin–Ciocalteu's reagent[7]. This reagent (diluted with 4 times its volume of water) was sprayed on to the dried paper, which was then placed a few minutes in an atmosphere saturated with ammonia, when blue spots appeared against a practically white background. The R_F values were determined and used for the calculation of $R_M = \log R_F/(1 - R_F)$.

RESULTS AND DISCUSSION

The results are summarized in Tables I–III. Table I shows R_F and R_M values of compounds with various substituents in the *p,p'*-position but with the alkylidene moiety unchanged:

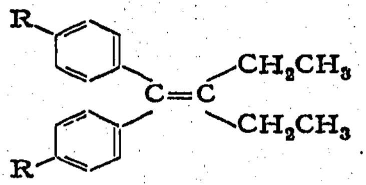


In Tables II and III, on the other hand, the alkylidene moiety is varied but the *p,p'*-diacetoxy-phenyl groups are kept constant. Both the alkylidene and the cycloalkylidene compounds give a linear correlation between R_M and the number of carbon atoms in the chain and ring, respectively (Figs. 2 and 3). The slopes of the different lines representing different mixtures of methanol and water decrease with increasing metha-

TABLE I

R_F AND R_M VALUES OF *p,p'*-SUBSTITUTED 1,1-DIPHENYL-2-ETHYLBUTENE-1 IN METHANOL–WATER SYSTEMS

Paper Whatman No. 1, impregnated with paraffin oil. 5 hours ascending technique. Every R_F value is a mean of 3–6 different chromatograms, excepting the values for the standard substance for which every R_F is a mean of about 30 determinations.

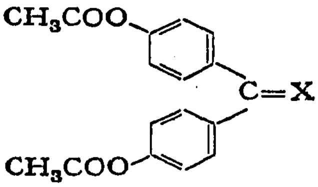


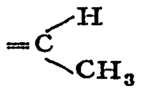
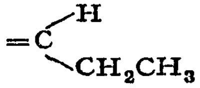
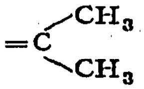
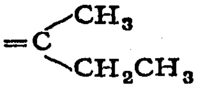
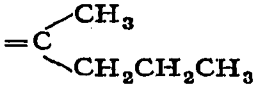
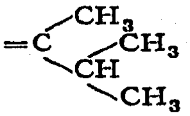
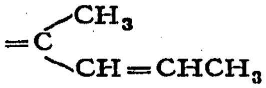
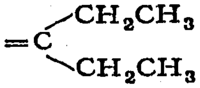
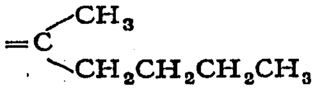
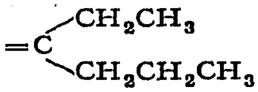
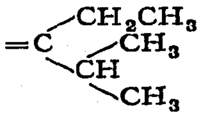
Compound No.	R	*Solvent systems, CH_3OH-H_2O* 1:1 R_F	1:1 R_M	1.5:1 R_F	1.5:1 R_M	2:1 R_F	2:1 R_M	3:1 R_F	3:1 R_M
10	H	0.00	$-\infty$	0.04	-1.37	0.14	-0.79	0.49	-0.02
10B	OH	0.72	$+0.41$	0.86	$+0.79$	0.90	$+0.95$	0.95	$+1.28$
10C	CH_3COO*	0.22	-0.55	0.55	$+0.09$	0.75	$+0.48$	0.88	$+0.87$
10D	CH_3CH_2COO	0.06	-1.20	0.24	-0.50	0.52	$+0.04$	0.81	$+0.63$
10F	$CH_3(CH_2)_5COO$	0.00	$-\infty$	0.00	$-\infty$	0.00	$-\infty$	0.05	-1.28
10E	$(CH_3)_3CCOO$	0.00	$-\infty$	0.03	-1.51	0.11	-0.91	0.46	-0.07
10A	CH_3O	0.02	-1.69	0.10	-0.95	0.29	-0.39	0.64	$+0.25$

* Standard. For the influence on R_F of varying the alkylidene moiety in this substance, see Tables II and III.

TABLE II

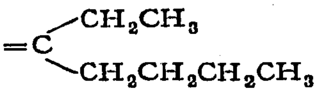
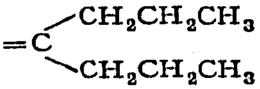
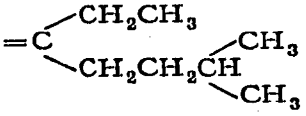
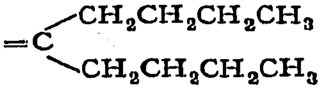
THE INFLUENCE ON R_F AND R_M VALUES OF DIFFERENT ALKYLIDENE CHAINS
(For further explanation, see Table I)



Compound No.	Alkylidene chain X	No. of C atoms in X	Solvent systems, CH_3OH–H_2O							
			1:1		1.5:1		2:1		3:1	
			R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M
2C	 $=C(H)CH_3$	2	0.62	+0.21	0.79	+0.58	0.89	+0.91	0.94	+1.20
3C	 $=C(H)CH_2CH_3$	3	0.44	—0.11	0.67	+0.31	0.83	+0.69	0.91	+1.01
4C	 $=C(CH_3)CH_3$	3	0.51	+0.02	0.76	+0.50	0.85	+0.75	0.93	+1.12
5C	 $=C(CH_3)CH_2CH_3$	4	0.36	—0.25	0.63	+0.23	0.80	+0.60	0.90	+0.95
6C	 $=C(CH_3)CH_2CH_2CH_3$	5	0.23	—0.53	0.52	+0.04	0.72	+0.41	0.89	+0.91
7C	 $=C(CH_3)CH(CH_3)CH_3$	5	0.18	—0.66	0.52	+0.04	0.73	+0.43	0.90	+0.95
8C	 $=C(CH_3)CH{=}CHCH_3$	5	0.22	—0.55	0.57	+0.12	0.76	+0.50	0.89	+0.91
10C*	 $=C(CH_2CH_3)CH_2CH_3$	5	0.22	—0.55	0.55	+0.09	0.75	+0.48	0.88	+0.87
9C	 $=C(CH_3)CH_2CH_2CH_2CH_3$	6	0.14	—0.79	0.42	—0.14	0.64	+0.25	0.86	+0.79
11C	 $=C(CH_2CH_3)CH_2CH_2CH_3$	6	0.15	—0.75	0.46	—0.07	0.66	+0.29	0.86	+0.79
111C	 $=C(CH_2CH_3)CH(CH_3)CH_3$	6	0.13	—0.83	0.46	—0.07	0.70	+0.37	0.87	+0.83
122C	 $=C(CH_3)CH_2CH_2CH_2CH_2CH_3$	7	0.08	—1.06	0.30	—0.37	0.56	+0.11	0.82	+0.66

(continued on p. 335)

TABLE II (*continued*)

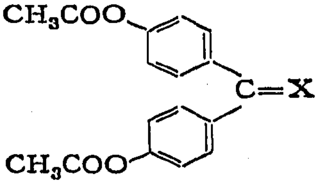
Compound No.	Alkylidene chain X	No. of C atoms in X	Solvent systems, CH_3OH-H_2O							
			1:1		1.5:1		2:1		3:1	
			R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M
121C		7	0.09	−1.01	0.37	−0.23	0.57	+0.12	0.79	+0.58
12C		7	0.11	−0.91	0.38	−0.21	0.62	+0.21	0.84	+0.72
131C		8	0.06	−1.20	0.28	−0.41	0.52	+0.04	0.80	+0.60
13C		9	0.03	−1.51	0.17	−0.69	0.39	−0.20	0.74	+0.45

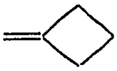
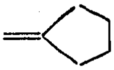
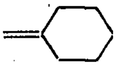
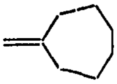
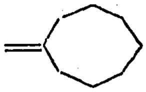
* Standard. For variation of the phenyl substituents, see Table I.

TABLE III

THE INFLUENCE OF DIFFERENT CYCLOALKYLIDENE RINGS ON R_F AND R_M VALUES

(For further explanation, see Table I)



Compound No.	Cycloalkylidene ring X	No. of C atoms in X	Solvent system, CH_3OH-H_2O							
			1:1		1.5:1		2:1		3:1	
			R_F	R_M	R_F	R_M	R_F	R_M	R_F	R_M
14C		4	0.35	−0.27	0.60	+0.18	0.76	+0.50	0.88	+0.87
15C		5	0.26	−0.45	0.50	±0	0.70	+0.37	0.85	+0.75
16C		6	0.18	−0.66	0.44	−0.11	0.63	+0.23	0.82	+0.66
17C		7	0.12	−0.87	0.36	−0.25	0.58	+0.14	0.79	+0.58
18C		8	0.08	−1.06	0.26	−0.45	0.48	−0.04	0.74	+0.45

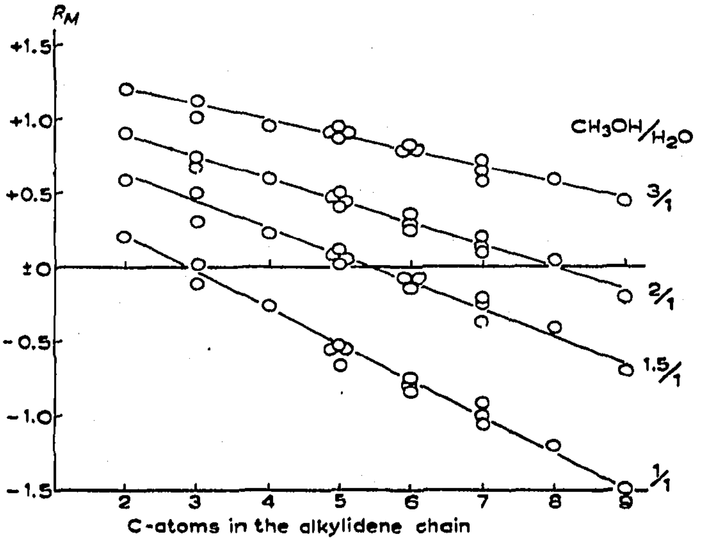


Fig. 2. Relation between the R_M values and the number of carbon atoms in the alkylidene chain of 1,1-bis-(*p*-acetoxyphenyl)-alkenes.

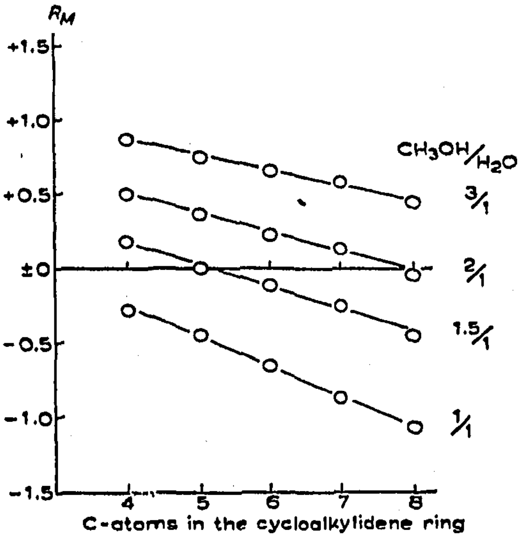


Fig. 3. Relation between the R_M values and the number of carbon atoms in the cycloalkylidene ring of 1,1-bis-(*p*-acetoxyphenyl)-cycloalkylidenemethanes.

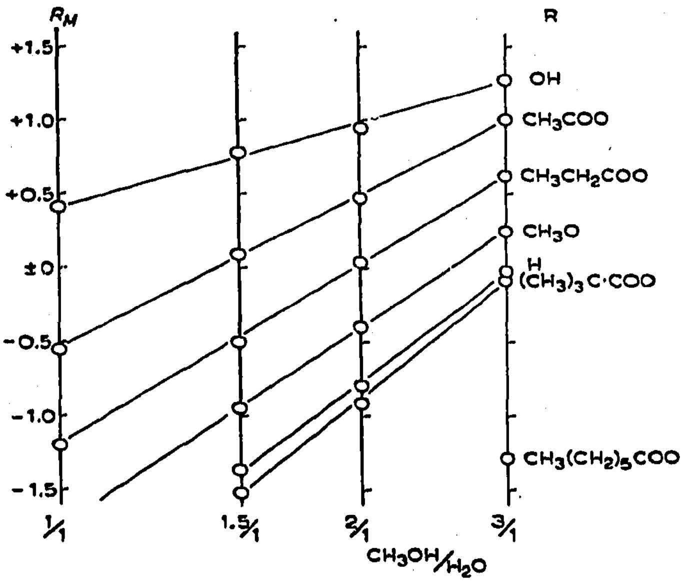
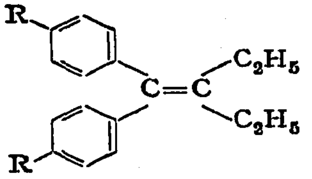


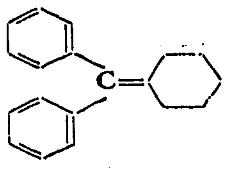
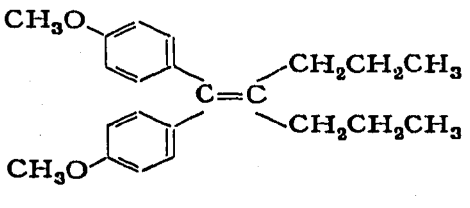
Fig. 4. Relation between the R_M values and the composition of the solvent system methanol–water for the *p,p'*-substituted compounds:



nol content of the system. This inverse relation is discussed by THOMA[8] in his paper on a graphical method for evaluating chromatographic partition parameters.

Fig. 4 shows the relation between R_M and the concentration of methanol in the mobile phase. Corresponding diagrams can also be constructed for all the other compounds studied. A linear relationship was invariably found between R_M and the concentration of methanol in the methanol–water system. The R_F value for similar substances can be easily predicted from the diagrams. A few examples are given in Table IV.

TABLE IV

Compound No.	*Formula*		*CH_3OH-H_2O*			
			1:1	*1.5:1*	*2:1*	*3:1*
16		R_F expected	0.00	0.03	0.08	0.30
		R_F observed	0.00	0.03	0.09	0.31
12A		R_F expected	0.00	0.05	0.18	0.48
		R_F observed	0.00	0.05	0.17	0.50

The system proved very useful for the purpose in view, but it is of only limited value for chromatography of contaminated solutions, such as urine extracts. For such impure mixtures it is preferable to use a system with a larger capacity such as formamide/toluene or propylene glycol/cyclohexane. It is remarkable that the paraffin oil/methanol–water system gives such a high ΔR_M for small changes in the size of the substituent, an effect not occurring to the same extent with more lipophilic paper, *e.g.* silicone treated or acetylated paper. No significant change in the R_M values was found on branching of (substances No. 7C, 111C, 131C), or introduction of a double bond into the alkylidene moiety (substance No. 8C).

SUMMARY

A series of *p,p'*-substituted 1,1-bis-(phenyl)-alkenes and -cyclo-alkylidenemethanes were studied chromatographically on slightly lipophilic paper and with methanol–water in varying proportions as the mobile phase. A linear correlation was found between the R_M value and the number of carbon atoms in the alkylidene and cycloalkylidene moieties. The relation between R_M and the concentration of methanol in the mobile phase was also found to be linear. With the aid of the R_M values determined, it was possible to predict the chromatographic characteristics of new substances in the series, which proved useful for identifying these substances and assessing their degree of purity.

REFERENCES

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