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STRUCTURE–RETENTION CORRELATION OF DIHYDROPYRAZOLO-TRAZINONE DERIVATIVES IN THIN-LAYER AND GAS CHROMATRO-GRAPHY

M. BIDLÓ-IGLÓY*, F. SZEDERKÉNYI and E. TIHANYI

Institute for Drug Research, P.O. Box 82, H-1325 Budapest (Hungary)

SUMMARY

Relationships between logarithmic retention factors and carbon atom numbers for some homologous series of the title compounds were studied in thin-layer and gas chromatographic systems. Reversed-phase R_M values and Kováts retention indices showed linear correlation with the carbon atom numbers of the homologous species. In silica gel chromatography, these correlations were non-linear in several cases. The chromatographic behaviour of the compounds investigated could be explained on the basis of their structural properties.

INTRODUCTION

It is known from the literature that linear relationships between the logarithmic retention factors and carbon atom numbers have been observed for many types of homologous series in gas chromatography (GC)[1], adsorption[2], partition[3] and reversed-phase partition chromatography[3,4]. These linear free-energy relationships (LFER) are most frequently manifested in linear plots of the logarithmic retention factors *versus* the number of carbon atoms in the alkyl substituents. However, they are not restricted to methylene groups[5,6].

Conformational, stereochemical and electron effects can cause deviations from linearity[6,7], but the concept of additivity of the logarithmic retention with the substituent groups is usually valid in chromatography and can be used for structure–retention investigations[8].

In a previous communication[9], the gas chromatographic behaviour of the title compounds was studied. The aim of this work was to investigate the thin-layer chromatographic (TLC) properties of these molecules on silica gel and "reversed" phases and to compare the results with those obtained in gas chromatography (GC).

R_M values and Kováts retention indices were used as logarithmic retention data and correlated with the carbon atom number of the substituents. Deviations from the linear relationships were observed in the case of the homologous series substituted on the N-5 nitrogen atom (see Scheme 1) on silica gel and in GC systems. Linear relationships were found between the reversed-phase R_M values and Kováts

indices. Relative retentions of isomeric pairs substituted on N-5 and N-6, respectively, were examined.

The compounds investigated are characterized by the following formulae:
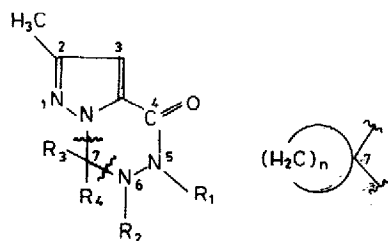
R_1, R_2 = H, alkyl

R_3, R_4 = H, Me, cycloalkyl (n = 4,5)

Scheme 1.

EXPERIMENTAL

TLC experiments were carried out on Kieselgel $60F_{254}$ (20×20 cm) and RP_{18} F_{254s} (10×20 cm) plates (Merck, Darmstadt, F.R.G.; Cat. No. 5715 and 15423, respectively). Development was performed with butyl acetate in the case of silica gel and methanol–water mixtures on the reversed-phase (RP) layers. Unsaturated and sandwich chambers were used for the developments, respectively. Spots were detected at 254 nm. R_F values were measured with a standard deviation of *ca.* ± 0.007. R_M values were calculated on the basis of the measured R_F values.

GC was carried out with a Hewlett-Packard 5720 A chromatograph with a single flame ionization detector (Hewlett-Packard). The glass (silanized) columns (2 m $\times$ 2 mm I.D.) contained 3% OV-101, OV-17 or OV-225 stationary phases on Gas Chrom Q 80–10 mesh (Applied Science Labs.). Nitrogen was used as the carrier gas at a flow-rate of 20 ml/min. The column temperature was 220°C and the injector and detector temperatures were 270°C.

The sample size was 1 μl (10-μl Hamilton syringe) from 10- or 20-mg/ml solutions in dry pyridine. The amplifier attenuations were 16 and 32 $\times$ 100. The recorder speed was 15 in./h. The retention times were measured with a stop-watch to a 0.1-s accuracy. Kováts retention indices[10] were determined as averages of three measurements, and the accuracy was calculated to be ± 2 index units[11].

Kováts retention indices and the R_M values of the compounds investigated are presented in Table I.

RESULTS AND DISCUSSION

It was found that the polarity order, characterized by R_F and R_M values, was a function of carbon atom number of the compounds for each chromatographic

TABLE I

KOVÁTS RETENTION INDICES AND R_M VALUES OF THE COMPOUNDS INVESTIGATED

Eluents: (1) methanol–water (7:3); (2) methanol–water (8:2); (3) n-butyl acetate.

Series	Compound*	Kováts indices (220°C)			R_M values		
					RP_{18}		Silica gel
		OV-101	OV-17	OV-225	Eluent		
					1	2	3
1							
$n = 5^*$; R_2 = methyl*							
R_1 = methyl	1a	1939	2302	2692	0.308	−0.058	−0.140
ethyl	1b	2006	2364	2739	0.477	0.070	−0.389
n-propyl	1c	2070	2405	2800	0.659	0.194	−0.525
n-butyl	1d	2160	2506	2871	0.826	0.308	−0.602
2							
R_1 = methyl*; R_2 = H*							
R_3 = R_4 = H	2a	1600	2014	2486	−0.389	−0.550	0.630
R_3 = R_4 = methyl	2b	1554	1899	2364	−0.308	−0.501	0.368
$n = 4$	2c	1842	2221	2721	−0.017	−0.308	0.122
$n = 5$	2d	1955	2370	2872	0.105	−0.213	0
3							
R_1 = H*; R_2 = methyl*							
R_3 = R_4 = H	3a	1544	1941	2536	−0.389	−0.602	0.689
R_3 = R_4 = methyl	3b**	1600	1956	2420			
$n = 4$	3c**	1853	2254	2735			
$n = 5$	3d	1983	2406	2905	0.052	−0.250	0
4							
R_1 = R_2 = methyl*							
R_3 = R_4 = methyl	4a	1552	1558	2202	−0.070	−0.327	0.269
$n = 4$	4b	1807	2121	2514	0.194	−0.122	0.017
$n = 5$	4c	1939	2302	2692	0.308	−0.058	−0.140

* For formulae see Scheme 1; the designated substituents are constant in the given series.
** Compounds 3b and 3c showed decomposition in the TLC experiments.

method used. Considering isomer pairs (2a and 3a, 2d and 3d, respectively, see Table I), there was little difference in polarity. The compounds containing a free amide group (R_1 = H) were more polar than the corresponding methyl analogues (R_1 = CH_3).

Some compounds of this group could be considered as members of a homologous series (see Table I). At first we studied those derivatives where n = 5 at C-7, R_2 = CH_3 and R_1 is a n-alkyl group with increasing carbon atom number (Series 1). Fig. 1 shows the R_M values as a function of the carbon atom number of R_1 varying between 1 and 4. It can be seen that in the case of reversed-phase chromatography, the R_M values, which can be considered as a measure of retention, show a linear
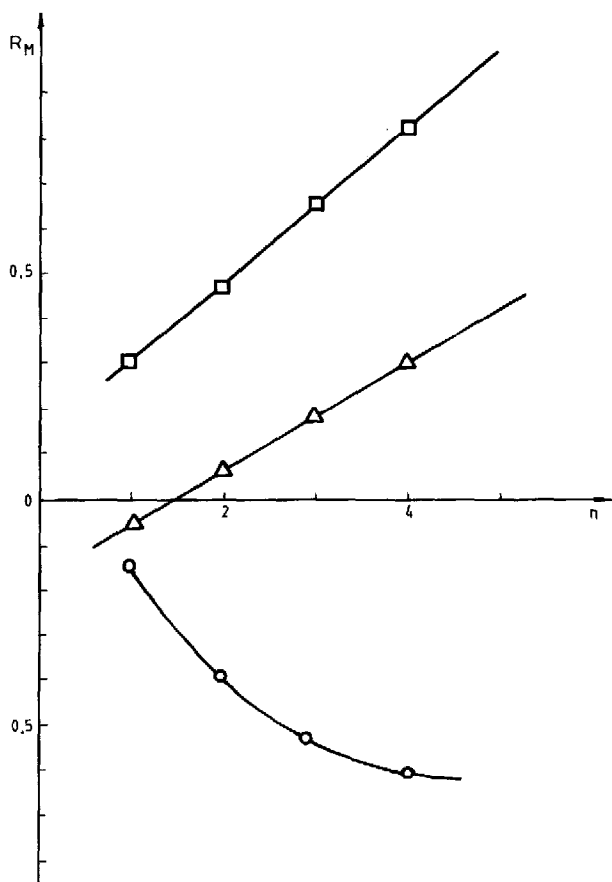
Fig. 1. Correlation between the R_M values and carbon atom number of the n-alkyl substituents in Series 1 (substitutions on N-5). (○) Silica gel, n-butyl acetate; (□) RP$_{18}$, methanol–water (7:3); (△) RP$_{18}$, methanol–water (8:2).

correlation with the carbon atom number. In silica gel chromatography, the retention decreases with the carbon atom number, but the correlation is not linear.

This difference between the two chromatographic methods can be explained on the basis of different mechanisms of retention. In reversed-phase chromatography, the extent of retention is an unambigous function of the alkyl chain-length. However, on silica gel, two effects are added. The most strongly adsorbed group in these molecules is the polar amide group ($R_1 = H$). This was demonstrated by the elution order of isomer pairs 3a,2a and 3d,2d (see Table I). In compounds substituted on their N-5 atoms by an alkyl group, the increasing electron density causes an increase in the basicity of the molecule and, as a consequence, an increase in the extent of retention. However, this effect proved to be slight. The stronger effect is the decrease of retention by steric hindrance of adsorption. The result of these phenomena is the decrease of retention as a function of alkyl chain-length, but this decrease is less with higher carbon atom numbers. A similar phenomenon was reported in earlier work[12] on the chromatography of N-alkylanilines.

TABLE II

GAS CHROMATOGRAPHIC RETENTION INDEX INCREMENTS FOR THE METHYLENE UNITS IN HOMOLOGOUS SERIES 1

$n(CH_2)$	Compound	Kováts retention index increments $(\delta I = I_{n+1} - I_n)$		
		I_{OV-101}	I_{OV-17}	I_{OV-225}
1	1a	—	—	—
2	1b	67	62	47
3	1c	63	51	61
4	1d	90	101	71

Gas chromatographic retention indices increments for the methylene units in Series 1 $(\delta I_{n+1,n})$ are demonstrated in Table II. It is appearent that δI_4 values are significantly higher than δI_3 and δI_2 on the apolar (OV-101) and moderately polar (OV-17) phases. This deviation can be explained by a conformational effect.

However, a linear relationship exists between the GC retention indices and the carbon atom number for 1a, 1b and 1c. Consequently, good linear correlation could be observed between the reversed-phase R_M values and the GC retention indices for these members of Series 1. Fig. 2 demonstrates this relationship. The values of cor-
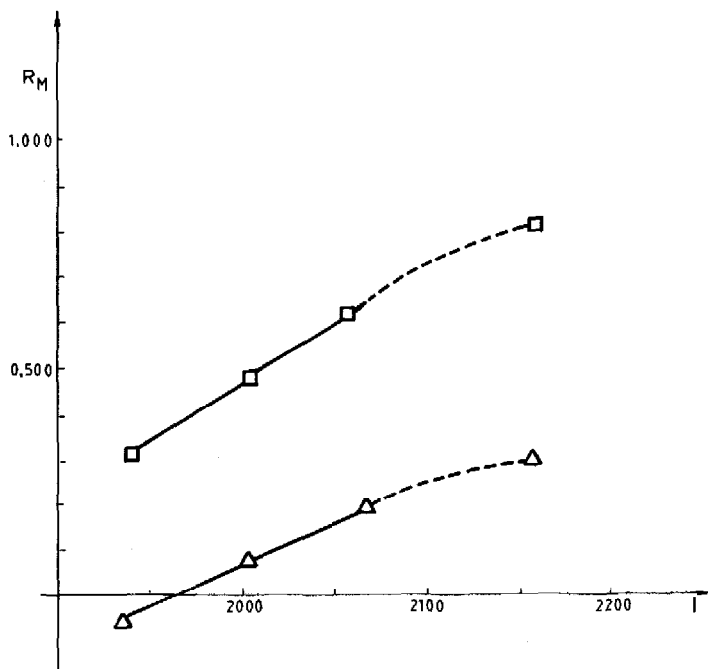

Fig. 2. Relationship between the reversed-phase R_M values and Kováts retention indices (OV-101, 220°C) for the homologous membres of Series 1. (□) RP$_{18}$, methanol–water (7:3); (△) RP$_{18}$, methanol–water (8:2).

relation coefficients were r = 0.9994 (m = 0.0026782, b = -4.88838) for methanol–water (7:3) and r = 0.9999 (m = 0.001924, b = -3.788) for methanol–water (8:2).

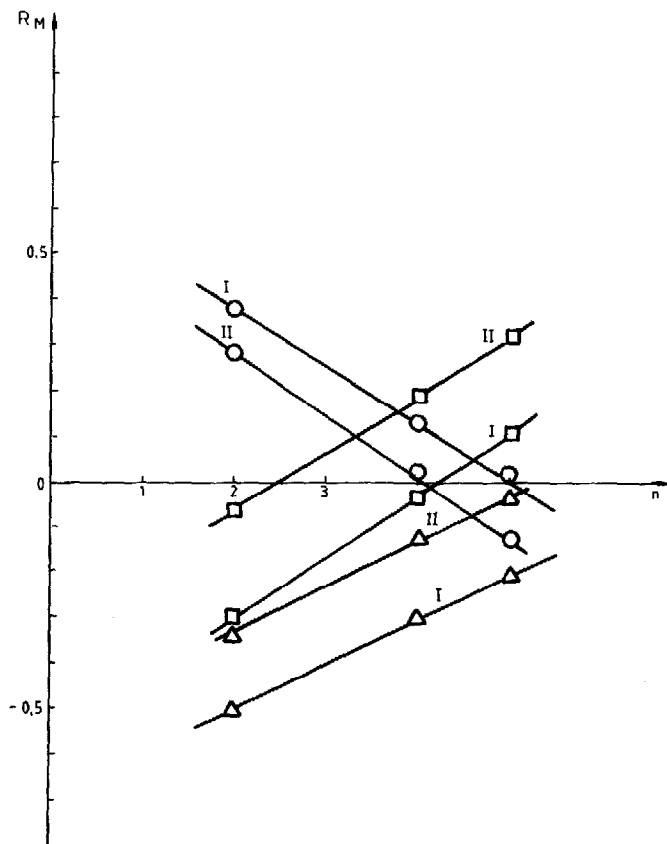Thin-layer chromatographic behaviour of two other homologous series (Series 2 and 3) is illustrated in Fig. 3.

Fig. 3. Thin-layer chromatographic behaviour of homologous Series 2 and 4 (substitutions on C-7) on silica gel and RP_{18}. (I) Series 2; (II) Series 4. ($\bigcirc$) Silica gel, n-butyl acetate; ($\square$) RP_{18}, methanol–water (7:3); ($\triangle$) RP_{18}, methanol–water (8:2).

These compounds differ in the substitution at the C-7 atom. The R_M values were plotted against the C-atom number of the alkyl (or cycloalkyl) groups attached to the C-7 atom. It can be seen that in the case of both series, a linear correlation was found in both silica and reversed-phase chromatography. Increasing carbon atom number went with a decrease in retention on silica gel and an increase in retention on reversed-phase plates. The decrease of R_M values was linear on silica gel too. The phenomenon illustrated in Fig. 1 was not observed in the case of N-5-substituted compounds.

Kováts indices were found to be in linear correlation with the carbon atom number of the alkyl (or cycloalkyl) group on C-7, as shown in Fig. 4 for Series 2, 3
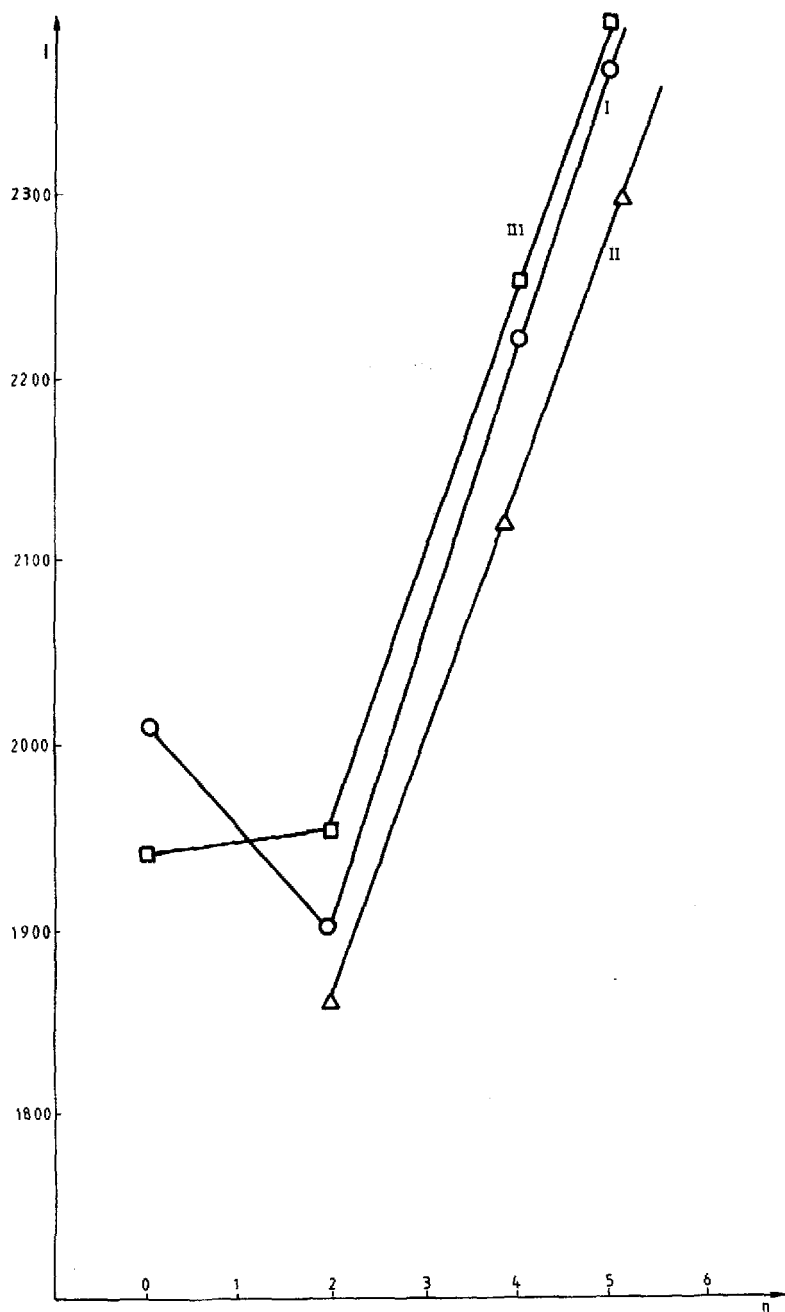
Fig. 4. Kováts retention indices (OV-17, 220°C) versus carbon atom number plots for homologous Series 2, 3 and 4, respectively. Methyl and cycloalkyl substitutions on C-7. (I) Series 2; (II) Series 4; (III) Series 3
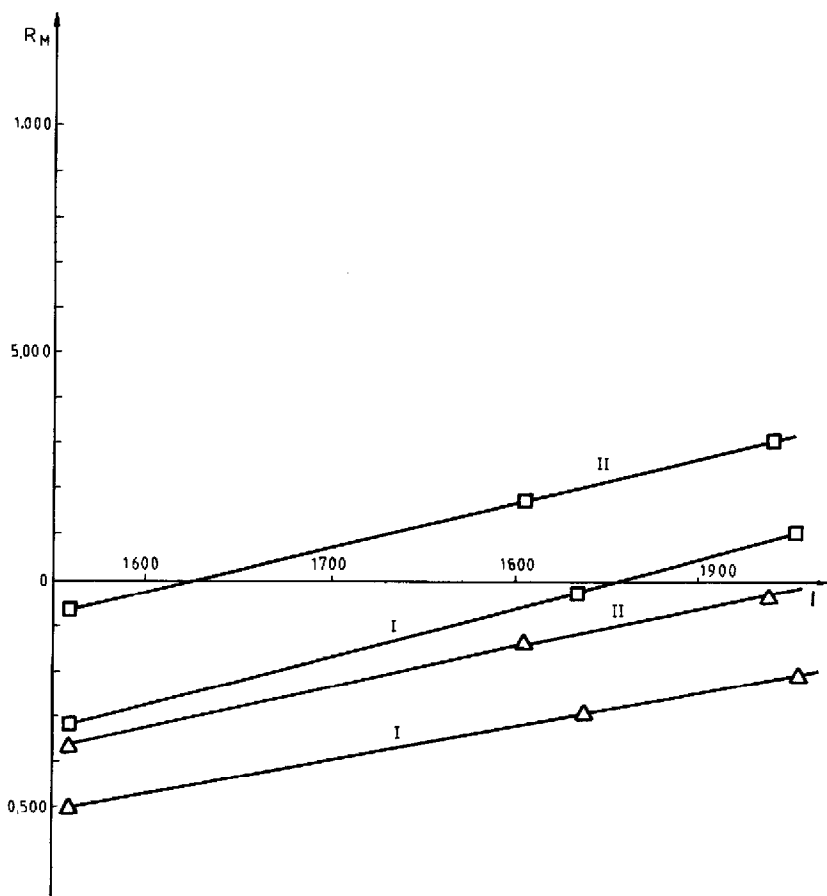
Fig. 5. Correlation between the reversed-phase R_M values and Kováts retention indices (OV-101) for Series 2 and 4. (□) RP_{18}, methanol–water (7:3); (△) RP_{18}, methanol–water (8:2). (I) Series 2; (II) Series 4.

and 4, respectively. Similarly, a good linear correlation exists between the reversed-phase R_M values and Kováts retention indices for these series. (Fig. 5 demonstrates the correlations for Series 2 and 4, respectively.)*

Fig. 4. shows that OV-17 is a selective stationary phase for the corresponding isomers of Series 2 and 3. It is also obvious that the unsubstituted species on C-7 ($R_3 = R_4 = H$; 2a and 3a) cannot be regarded as first members of the corresponding homologous Series 2 and 3.

The elution order for the isomeric pairs, except compounds 2a and 3a, is similar to that in TLC.

In Series 2 and 3, the compounds containing a methyl group on N-6 (Series 3) proved to be more polar than the corresponding isomer with the methyl group on N-5. However, the mobile phases used in the cases of homologous Series 2 and 4 (see

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* The values of correlation coefficients ranged between 0.993 and 0.9999.

TABLE III

R_F VALUES OF ISOMERIC PAIRS

Compound	R_F value	
	Silica gel (chloroform–methanol, 9:1)	RP_{18} (acetonitrile–water, 3:2)
2a	0.62	0.73 } no separation
3a	0.55	0.73
2d	0.87	0.43
3d	0.76	0.52

Figs. 1 and 3, Table I) gave a poor separation. Better separation of the corresponding isomeric pairs (2a,3a and 2d,3d) was achieved when the mobile phase was chloroform–methanol (9:1) on silica gel (unsaturated chamber) and acetonitrile–water (6:4) on RP_{18}, respectively. The R_F values are shown in Table III.

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