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## THIN-LAYER CHROMATOGRAPHY OF HOMOPYRIMIDAZOLE DERIVATIVES

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### SUMMARY

The chromatographic behaviour of 14 homopyrimidazole derivatives has been studied on thin layers of silica gel, aluminium oxide and ammonia-saturated silica gel. The relationships between structure and chromatographic behaviour, as well as the most suitable systems for separating the individual derivatives, are discussed on the basis of  $R_F$  values. The  $\Delta R_M$  values, showing the effect on the polarity, are given for certain functional groups.

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### INTRODUCTION

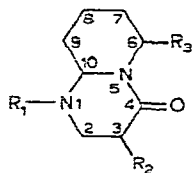
Many homopyrimidazole derivatives recently synthesized by Mészáros and co-workers<sup>1,2</sup> are biologically active. The compound designated MZ-144, an analgesic, has been marketed under the trade-name Probon. Both research and quality-control aspects have therefore justified studies on the adsorption thin-layer chromatographic behaviour of these compounds.

### EXPERIMENTAL

#### *Model compounds*

The homopyrimidazole (HPM) derivatives studied are listed in Table I (hereinafter, the compounds will be referred to only by their serial numbers in this table); their purities were controlled by elementary analysis and melting point tests<sup>2</sup>.

TABLE I  
STRUCTURE OF THE HOMOPYRIMIDAZOLE DERIVATIVES TESTED



Compound No.	Functional groups			
	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Δ
1	H	COOC <sub>2</sub> H <sub>5</sub>	H	—
2	H	COOC <sub>2</sub> H <sub>5</sub>	CH <sub>3</sub>	—
3	H	COOC <sub>2</sub> H <sub>5</sub>	H	2
4	H	COOC <sub>2</sub> H <sub>5</sub>	CH <sub>3</sub>	2
5	CH <sub>3</sub>	COOC <sub>2</sub> H <sub>5</sub>	CH <sub>3</sub>	2
6	—	COOC <sub>2</sub> H <sub>5</sub>	CH <sub>3</sub>	2, 10 (1)
7	—	COOC <sub>2</sub> H <sub>5</sub>	CH <sub>3</sub>	2, 10 (1)
8	—	H	CH <sub>3</sub>	2, 10 (1)
9	—	COOH	CH <sub>3</sub>	2, 10 (1)
10*	CH <sub>3</sub>	COOC <sub>2</sub> H <sub>5</sub>	CH <sub>3</sub>	2, 10 (1)
11	—	COOC <sub>2</sub> H <sub>5</sub>	—	2, 6, 8, 10 (1)
12	—	COOC <sub>2</sub> H <sub>5</sub>	CH <sub>3</sub>	2, 6, 8, 10 (1)
13	—	C <sub>6</sub> H <sub>5</sub>	—	2, 6, 8, 10 (1)
14	—	C <sub>6</sub> H <sub>5</sub>	CH <sub>3</sub>	2, 6, 8, 10 (1)
15*	CH <sub>3</sub>	COOH	CH <sub>3</sub>	2, 10 (1)
16*	CH <sub>3</sub>	—	CH <sub>3</sub>	2, 10 (1)

\* Anion: methylsulphate. Compound 10 is Probon, compound 15 its hydrolysis product, and compound 16 its decarboxylation product.

### Reagents

Unless the quality is specifically indicated, the reagents used were of analytical grade. The chromatographic developing solvents were specially purified<sup>3</sup>, the fraction of appropriate boiling point being used.

### Chromatographic procedure

A 0.25- $\mu$ m layer of Kieselgel GF<sub>254</sub> (Merck) or Alumina GF<sub>254</sub> (Merck), prepared by using a Desaga spreader, was activated at 110° for 1 h. Development was for 10 cm, at a temperature of 22  $\pm$  2°, in a tank lined with filter paper and pre-saturated for 1 h. The spots were marked under UV radiation: UV-inactive spots were detected by spraying with 0.1 N potassium permanganate (yellow spots on a pink background).

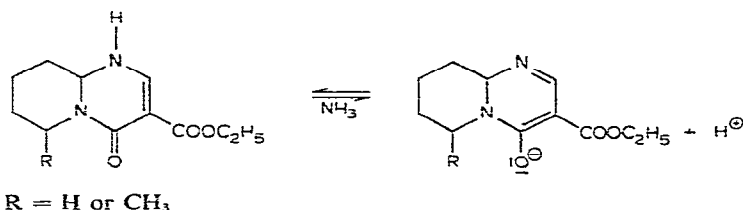
### RESULTS

It was assumed that aluminium oxide and silica gel layers would be selective for the HPM derivatives because of the acidic or basic properties of these compounds. Since numerical dissociation data were available only for Probon<sup>4</sup> (viz., pK<sub>b1</sub> = 8.65; pK<sub>b2</sub> = 22.22; and pK<sub>a</sub> = 10.69), it was expected that the modifying effect of indi-

vidual substituents on the electron distribution and acidity or basicity of the compounds could be deduced from the sequence of (or difference between)  $R_F$  values. The  $R_F$  values in Tables II–V represent the averages of not less than three parallel developments performed on different plates.

#### Results with a "basic" silica gel layer

The results of developments on silica gel in an atmosphere of ammonia<sup>5</sup> and with methanol as solvent are shown in Table II. The atmosphere was not saturated in the usual way by pouring only the developing solvent on the bottom of the tank, but a beaker containing 25% ammonia solution was simultaneously placed in the tank. It is assumed that a polar and basic ammonium silicate layer containing ammonia is formed during the saturation, and that the compounds migrate in this layer according to their relative acidities. This assumption seems to be confirmed by Table II, which shows that the 3-carboxylic acid derivative (compound 9) is very strongly bound in this system. The  $\Delta^2$ -4-one derivatives with no substituent at N<sup>1</sup> (compounds 3 and 4) also exhibit very strong adsorption. To interpret this latter observation, we assumed that these compounds "react" in their enolic forms, under the given conditions, thus:



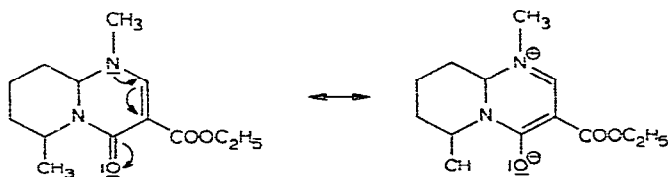
A methyl group in position C<sub>6</sub> increases the electron density around the oxygen bound to C<sub>4</sub>, thereby inhibiting enolisation, so that the  $R_F$  value of compound 4 is higher (see Table III).

TABLE II

$R_F \times 100$  VALUES OF HPM DERIVATIVES IN THE SYSTEM SILICA GEL-AMMONIA-METHANOL

Compound No.	$R_F \times 100$
1	37
2	43
3	0
4	0
5	21
6	40
7	51
8	52
9	0
10	46
11	46
12	61
13	69
14	73

The substitution of a methyl group at N<sup>1</sup> excludes the possibility of enolisation. Thus, it can be assumed that compound 5 reacts with a zwitterion structure and is adsorbed as a salt-like compound, *viz.*



The completely saturated derivatives (compounds 1 and 2) behave like "pure" ketones, *i.e.*, their  $R_F$  values are significantly higher than those of the  $\Delta^2$ -derivatives, the enol forms of which are adsorbed. The presence of the  $\Delta^{10(1)}$ -bond (compounds 6 and 7) considerably increases the  $R_F$  value, because the conjugated  $\pi$ -bond system favours aromatization and decreases the electron density around the oxygen bound to C<sub>4</sub>. The strength of adsorption is found between the carbonyl and aromatic hydrocarbon compounds. This effect is further increased if the second ring is also unsaturated (compare the  $R_F$  values of compounds 6 and 11 or compounds 7 and 12).

#### Results with an aluminium oxide layer

The conclusions drawn in connection with the ammoniacal silica gel system are mostly valid for development with chloroform-methanol mixtures on an aluminium oxide layer (see Table III); however, the sequence of the  $R_F$  values is sometimes changed. For example, Probon (compound 10) is adsorbed more strongly to aluminium oxide than are the completely saturated compounds 1 and 2 or the  $\Delta^{2,10(1)}$ -unsaturated compound 6. Also, the difference between the  $R_F$  values of Probon and "nor-Probon" (compound 7) is considerably higher than was expected.

TABLE III

$R_F \times 100$  VALUES OF HPM DERIVATIVES ON ALUMINIUM OXIDE LAYER

Compound No.	Chloroform	Chloroform-methanol					Methanol	
		99:1	98:2	95:5	90:10	50:50		80:20
1	6	58	88	90	100	89	83	83
2	8	61	91	97	100	88	85	84
3	0	0	0	29	48	70	56	50
4	0	0	0	32	58	75	60	55
5	0	8	28	70	89	80	64	56
6	7	56	86	97	100	87	82	80
7	12	67	94	97	100	88	82	81
8	23	71	95	100	100	86	82	80
9	0	0	0	0	0	0	0	0
10	0	30	65	89	95	84	74	70
11	8	53	82	95	100	85	79	78
12	16	70	92	96	100	86	81	79
13	45	87	100	100	100	86	81	78
14	52	90	100	100	100	86	82	80

*Results with a silica gel layer*

Developments with chloroform-methanol mixtures were also carried out on silica gel layers; the results are shown in Table IV.

TABLE IV

 $R_F \times 100$  VALUES OF HPM DERIVATIVES IN SILICA GEL LAYER

Compound No.	Chloroform	Chloroform-methanol						Methanol
		99:1	98:2	95:5	90:10	50:50	20:80	
1	0	0	16	51	77	74	45	36
2	0	3	29	53	86	76	20	14
3	0	0	0	2	16	43	63	63
4	0	0	0	0	20	45	58	51
5	0	0	7	18	40	44	56	51
6	0	2	22	51	73	73	23	20
7	0	2	28	62	79	78	65	65
8	0	4	11	44	76	73	20	16
9	0	0	3	4	6	14	53	52
10	0	2	47	79	95	68	60	57
11	0	6	18	54	80	72	57	57
12	0	11	23	62	83	76	64	64
13	4	18	41	73	88	79	66	66
14	6	26	51	80	90	80	15	14

The selective effect of silica gel is more marked with certain pairs of compounds, e.g., the difference between the  $R_F$  values is higher for the  $\Delta^{2,10(1)}$  ("nor") and  $C_6$ -methyl derivatives (compounds 7 and 6). It is interesting to note that the sequence of  $R_F$  values is inverted in the two layers for Probon (compound 10) and its  $N^1$ -"nor"-derivative (compound 7). The ionic compound (Probon) is much more strongly adsorbed to the aluminium oxide, while the opposite is true with silica gel for many solvent mixtures.

The  $R_F$  values show first an increase, then a decrease as the methanol content of the developing solvent increases. This indicates (in agreement with our former experience) adsorption of methanol as well as the effect of a partition mechanism. In a few instances, these factors result in an enhanced selective capacity, and they produce inversion of the  $R_F$  sequence for many pairs of compounds.

Other mixed developing solvents were tested in order to study the adsorption mechanism of HPM derivatives and the selective effect of the various solvents. Table V shows that the order of  $R_F$  values roughly follows the sequence of the dielectric constants of the solvent mixtures. This indicates that none of the solvents tested selectively interacts with the HPM derivatives. It is therefore not surprising that none of the systems exhibits particular selective power.

*Double-spot formation of Probon and its separation from other HPM derivatives*

It has been observed that the behaviour of Probon differs in one respect from that of all the other HPM derivatives tested. When an aqueous solution of Probon is developed, double-spot formation occurs. If the pH of the test solution is increased,

TABLE V

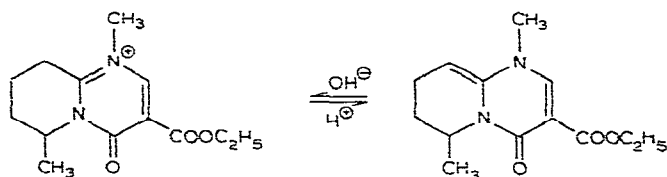
$R_F \times 100$  VALUES OF HPM DERIVATIVES ON ALUMINIUM OXIDE LAYER WITH VARIOUS BINARY DEVELOPING SOLVENTS

Compound No.	Developing solvent			
	Chloroform-benzene (90:10); $\epsilon^* = 4.30$	Chloroform-ether (60:40); $\epsilon^* = 5.51$	Chloroform-ethyl acetate (50:50); $\epsilon^* = 6.41$	Chloroform-methyl ethyl ketone (99:5); $\epsilon^* = 5.33$
1	10	12	31	15
2	33	36	43	27
3	0	0	0	0
4	0	0	0	0
5	0	0	5	0
6	11	11	26	17
7	13	25	47	22
8	24	34	55	41
9	0	0	0	0
10	0	6	23	0
11	8	7	29	12
12	10	21	49	28
13	49	55	74	67
14	58	66	79	77

\*  $\epsilon$  = dielectric constant of mixture.

the quantity of material in the second spot is also increased, as can be seen from Fig. 1.

We assume that the tautomeric form of Probon, *viz.*,



migrates in the second spot; this assumption is supported by the fact that, when, after development, the isolated spot is eluted with 0.1 *N* hydrochloric acid, the eluate shows absorption max. at 286 and 234 nm, wavelengths characteristic of the spectrum of Probon. If, on the other hand, the pH of the eluate is adjusted to 6.2, the maximum appears at 264 nm. A solution of untreated Probon in hydrochloric acid or sodium hydroxide exhibits behaviour identical with that of the corresponding eluate.

#### Separation of Probon from other HPM derivatives

Probon is intermediate among the HPM derivatives tested as far as polarity and migration rate are concerned. It is considerably less polar and acidic than the  $\Delta^2$ -derivatives, and it migrates more rapidly than do these derivatives. Owing to its quaternary ammonium salt character, Probon is more polar than the other  $\Delta^{2,10(1)}$ -

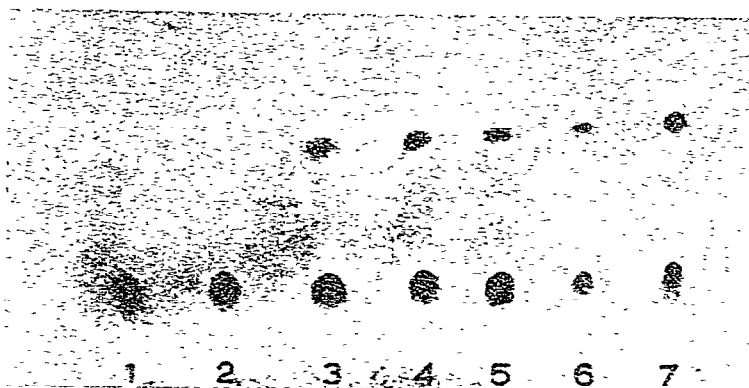


Fig. 1. Chromatograms of Probon solutions at various pH values. 1, pH 3.0; 2, pH 3.4; 3, pH 4.1; 4, pH 4.5; 5, pH 5.0; 6, pH 5.5; 7, pH 6.0.

derivatives, and much more polar than the  $\Delta^{2,6,8,10(1)}$ -derivatives; its migration rate is usually higher, and it can be separated from these compounds in appropriate systems.

The most probable impurities of Probon are its hydrolysis product and its decarboxylated derivative (compounds 15 and 16 in Table I), each of which can be detected (in concentrations down to 0.1%) in the presence of Probon by two-dimensional thin-layer chromatography. A typical chromatogram is shown in Fig. 2. A volume equivalent to 500  $\mu\text{g}$  of Probon and 0.5  $\mu\text{g}$  each of compounds 15 and 16 was applied as an ethanolic solution. The first development was performed with methanol in an atmosphere of ammonia, and chloroform-methanol (1:1) was used in the second dimension; the spots were marked under UV radiation. It can be seen that the two "impurity" compounds could be detected.

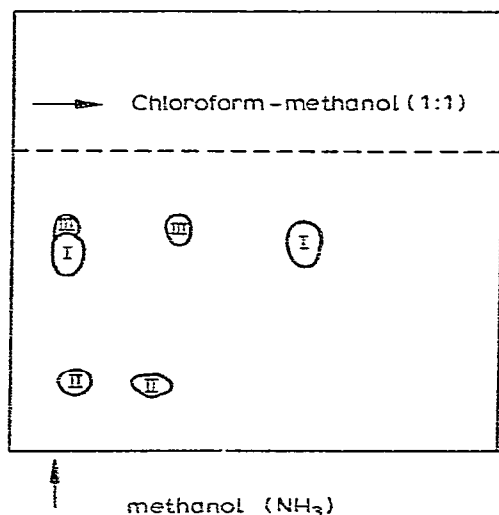


Fig. 2. Separation of Probon and its decomposition products by two-dimensional development. I = Probon (500  $\mu\text{g}$ ); II = its hydrolysis product (compound 15) (0.5  $\mu\text{g}$ ); III = its decarboxylated product (compound 16) (0.5  $\mu\text{g}$ ). Layer, silica gel.

TABLE VI

 $R_{M'}^0$  VALUES OF SOME FUNCTIONAL GROUPS OF HPM DERIVATIVES

The solvents used for development were as follows: 1 = chloroform-benzene (90:10); 2 = chloroform-ether (60:40); 3 = chloroform-ethyl acetate (50:50); 4 = chloroform-methyl ethyl ketone (95:5); 5 = chloroform-methanol (99:1); 6 = chloroform-methanol (98:2); 7 = chloroform-methanol (50:50); 8 = methanol; 9 = acetone.

Functional group	Derivative*	Development on aluminum oxide with solvent									Development on silica gel with solvent								
		1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
C <sub>6</sub> -methyl	OHPM	-0.65	-0.62	-0.21	-0.32	-0.05	-0.33	-0.05	-0.04	-0.12	-0.65	-0.62	-0.21	-0.32	-0.05	-0.33	-0.05	-0.04	-0.12
	HHPM																		
	THPM	-0.08	-0.43	-0.40	-0.14	-0.20	-0.14	-0.14	-0.12	0.00	-0.08	-0.43	-0.40	-0.14	-0.20	-0.14	-0.12	0.00	-0.40
	HPM	-0.11	-0.55	-0.37	-0.46	-0.32	-0.13	-0.13	-0.09	-0.13	-0.11	-0.55	-0.37	-0.46	-0.32	-0.13	-0.09	-0.13	-0.10
N <sup>1</sup> -methyl	THPM	1.17**	0.72*	0.47	1.45**	0.68	-0.36	0.22	0.29	1.59	1.17**	0.72*	0.47	1.45**	0.68	-0.36	0.22	0.29	1.59
	OHPM	1.04**	0.13**	1.65**	1.24**	2.14**	1.88**	0.58	0.95	1.86	1.04**	0.13**	1.65**	1.24**	2.14**	1.88**	0.58	0.95	1.86
Z <sup>1</sup>	C <sub>6</sub> -methyl-OHPM	1.69**	1.75**	1.87**	1.56**	2.19**	1.61**	0.59	0.87	2.07	1.69**	1.75**	1.87**	1.56**	2.19**	1.61**	0.59	0.87	2.07
	HHPM	-1.09**	-1.09**	-1.54**	-1.31**	-2.10**	-1.45**	-0.74	-1.83		-1.09**	-1.09**	-1.54**	-1.31**	-2.10**	-1.45**	-0.74	-1.83	

\* HPM = 3-ethoxycarbonyl-4-oxohomopyrimidazole; THPM = 6,7,8,9-tetrahydrohomopyrimidazole; HHPM = 1,5,7,8,9,10-hexahydrohomopyrimidazole; OHPM = 2,3,4,6,7,8,9,10-octahydrohomopyrimidazole.

\*\* Calculation based on value of 1 for  $R_f \times 100$ .



*Effect of a phenyl substituent*

The  $R_F$  value is significantly increased when a phenyl group replaces the ethoxycarbonyl group in an HPM derivative (compare the  $R_F$  values of compounds 11 and 13 or 12 and 14, respectively), the increase being large in certain systems. This phenomenon can be explained by the fact that the negative mesomeric effect of the phenyl group is stronger than that of the ethoxycarbonyl group. Thus, the phenyl group reduces the electron density around  $C_4$  much more than does the ethoxycarbonyl group.

*Determination of the  $\Delta R_{M_g}$  values*

The  $\Delta R_{M_g}$  values<sup>6</sup> of some functional groups in various developing-solvent mixtures (see Table VI) were calculated in order further to clarify the relation between the structure and chromatographic behaviour:

$$\Delta R_{M_g} = R_{M_1} - R_{M_2}$$

where  $R_{M_1}$  is the  $R_M$  value of the HPM derivative with the substituent being investigated, and  $R_{M_2}$  is the  $R_M$  value of the unsubstituted molecule.

An  $R_F$  value of 0 was obtained with some compounds: in such instances (shown by a double asterisk in Table VI), the value of  $R_F \times 100$  was taken as 1 and that of  $R_M$  as 1.996 for the calculations; it is evident that these data indicate only the character of the change in polarity.

Table VI shows that the presence of the  $C_6$ -methyl group decreases the polarity and the adsorption power in each of the systems studied, while the introduction of an  $N^1$ -methyl group into the THPM molecule increases the polarity and adsorption power in all but one system. The introduction of the  $\Delta^2$ -bond increases the polarity of OHPM derivatives, whereas the formation of  $\Delta^{2,10}$ -conjugation produces the opposite effect.

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