CHROMATOGRAPHY OF PYRAZOLE DERIVATIVES ON ACETYLATED PAPER

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INTRODUCTION

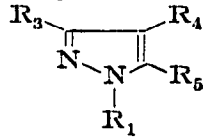
Good methods of separation and identification are absolutely necessary for chemists dealing with pyrazole ring systems. A literature search yielded only an indication of chromatographic separation of 2-substituted 5-pyrazolones[1] which differ considerably in structure by at least one substituent and in molecular weight. The common solvents used in the chromatography of the imidazoles were of no use for pyrazoles. We previously found some solvent systems suitable for the paper chromatography of pyrazole-carboxylic acids[2] (see Table I) and aminopyrazoles[3]. But for

TABLE I

CHROMATOGRAPHY OF PYRAZOLE-CARBOXYLIC ACIDS

	*Substituents**				*R_F values***
	R_1	R_3	R_4	R_5	
1	H	CH_3	H	COOH	0.22
2	CH_3	CH_3	COOH	CH_3	0.27
3	iso-C_5H_{11}	C_4H_9	H	COOH	0.79
4	C_6H_5	H	H	COOH	0.40
5	C_6H_5	H	COOH	H	0.33
6	H	C_6H_5	H	COOH	0.35
7	C_6H_5	CH_3	H	COOH	0.47
8	C_6H_5	COOH	H	Cl	0.48

* Numbering of the substituents in the pyrazole ring for this table and all subsequent tables is as follows:



** Solvent system: *tert.*-butanol–petroleum ether–2 *N* ammonia (25:5:2).

many pyrazoles, because of their low solubility in water, or because of difficulty of detecting chromatographic quantities, a paper chromatographic method could not be developed.

Pyrazoles that do not have an acidic function were separated and identified in almost all cases by thin-layer chromatography on Al_2O_3[3,4] (see Table II). But in

* Visiting fellow (Brazil).

this case, condensed systems or those with a strongly polar group could not be separated because of very low R_F values.

In the present work, we found that chromatography on acetylated paper gives a general method for the separation and identification of pyrazole compounds. This method can be used to control the direction and course of relatively complicated reactions. Acetylated paper permits the chromatography of significantly more substance than ordinary paper. In our case, with the same paper acetylated, the optimal quantity rises from 15–30 μg to 50–100 μg, making the identification of impurities with small R_F differences easier.

TABLE II

THIN-LAYER CHROMATOGRAPHY OF PYRAZOLE DERIVATIVES

	Substituent				*R_F values**		
	R_1	R_3	R_4	R_5	*1*	*2*	*3*
1	CH_3	CH_3	H	H	0.52	0.48	—
2	CH_3	H	H	CH_3	—	0.41	—
3	H	CH_3	H	CH_3	0.05	0.03	—
4	C_3H_7	CH_3	H	CH_3	0.55	0.49	—
5	C_5H_{11}	CH_3	H	CH_3	0.61	0.59	—
6	C_8H_{17}	CH_3	H	CH_3	0.67	0.66	—
7	$C_{16}H_{33}$	CH_3	H	CH_3	0.73	0.69	—
8	C_6H_5	H	H	H	0.69	0.71	—
9	C_6H_5	CH_3	H	CH_3	0.59	0.64	—
10	CH_2–C_6H_5	CH_3	H	CH_3	0.78	0.75	—
11	H	CH_3	NO	CH_3	0.01	0.03	—
12	CH_2–C_6H_5	CH_3	NO	CH_3	0.52	0.43	—
13	C_5H_{11}	CH_3	NO	CH_3	0.53	—	—
14	H	CH_3	$COCH_3$	CH_3	0.00	—	0.04
15	H	CH_3	COC_6H_5	CH_3	0.03	—	0.03
16	CH_3	CH_3	$COCH_3$	CH_3	0.27	—	0.49
17	CH_3	CH_3	COC_3H_7	CH_3	0.35	—	0.54
18	C_6H_5	CH_3	$COCH_3$	CH_3	0.43	—	0.66
19	C_6H_5	CH_3	H	H	0.70	—	0.84
20	C_5H_{11}	CH_3	COC_6H_5	CH_3	0.44	—	0.73

* Solvent systems: (1) benzene–chloroform (1:1); (2) petroleum ether–chloroform (1:1); (3) petroleum ether–chloroform (1:5).

EXPERIMENTAL

Chromatographic paper "Fast", from Volodarskii Factory (Leningrad), corresponding approximately to Whatman No. 1, was acetylated by ZIJP's[5]* method. Our technique of chromatographic separation was based on the method described by KŐSTIŘ AND SLAVIK[6]. As in ordinary paper chromatography[2], we worked with a battery of test tubes 250 × 25 mm. Only in certain cases, when the R_F values of the compounds to be separated were very close to each other or when we had a mixture of unknown substances to identify, did we use paper strips 35 cm long. The solvents used were the same as in ref. 6.

* In the abstract there is a misprint: 100 ml of toluene instead of 1000 ml in the original paper.

On the bottom of the test tubes we placed some solvent of the stationary phase ($CHCl_3$, C_6H_5Cl) and after spotting the substance on the starting point of the acetylated paper strip, we let it hang in the closed test tube, without touching the surface of the solvent, for 10–15 min, and then transferred it to another test tube containing the development solvent (moving phase), generally 80 % ethyl alcohol. In this test tube we attached to the cork stopper a little piece of cotton-wool, and immediately before beginning the development this was wetted with a few drops of the stationary phase. Development for 10–12 cm takes 15–20 min, and the complete operation takes 30–50 min. If *n*-octanol, which solves cellulose acetate but does not dissolve it, is employed as stationary solvent the acetylated paper strip is immersed in a 5 % solution of *n*-octanol in methanol for 30 min and then dried in air for 50–60 min[6].

With a long strip the technique is as follows: 50 ml of stationary solvent and an empty Petri dish were placed on the bottom of a cylindrical chromatographic chamber 45–50 cm high. The strip is spotted at the starting points, dried and hung in the solvent vapour for 20–25 min. 30 ml of development solvent is poured into the Petri dish and the chromatograph is run until the solvent front rises approx. 30 cm. This takes, at 18–20°, with ethyl alcohol as development solvent, 2.5–3 h, a relatively short time as compared with ordinary paper chromatography. The sample should be dissolved in alcohol, methanol or other solvent inert to cellulose acetate[6].

Spots were identified with the help of U.V. light from an "Ultraquimiscope UI-1" source, with filters "UFS-1" (U.S.S.R.) or with iodine vapour[7,8] (from crystals in a closed chamber) or chlorine[8]. As the acetylated paper is hydrophobic and soluble in many organic solvents, the identification of spots with reagent solutions is limited compared with ordinary filter paper. Some coloured substances, dyes, etc., are visible on the paper and need no special identification method.

RESULTS

The results given in Tables III-V clearly show the versatility of the method for different pyrazole systems, from alkyl-aryl-pyrazoles (Table III) to azopyrazolone dyes (Table IV) and bi-heterocyclic pyrazole compounds with fused rings (Table V).

In Tables III and IV we see the dependence of the R_F value on the nature and position of the substituents. For derivatives of 1-phenyl-3-methylpyrazole, with the same solvent system ($CHCl_3$–80 % C_2H_5OH), the effect of the substituent in position 5 of the pyrazole ring on the R_F value is as follows: CH_3 R_F 0.18 (Table III, No. 4); NH_2 R_F 0.26 (Table III, No. 13); OH R_F 0.45 (Table IV, No. 2); COOH R_F 0.54 (Table IV, No. 22); SH R_F 0.70 (Table IV, No. 6). Such a succession and approximately similar sorts of R_F values are seen for other 1-phenylpyrazoles with other substituents, and other solvent systems.

A CH_3 group on position 3 of the pyrazole ring has in general a weak influence on the R_F value of the compound; but this influence depends on the presence of other substituents. As can be seen from Table III (No. 12 and 13) it is insignificant for 1-phenyl-5-aminopyrazoles, but increases significantly for 1-phenyl-5-pyrazolones (Table IV, No. 1 and 2), for 1-phenyl-5-carboxylic acids (Table IV, No. 21 and 22) and 1-phenyl-5-chloropyrazoles (Table III, No. 5 and 6).

For the 5-pyrazolones this may be explained by the greater influence of the

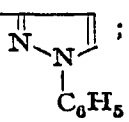
TABLE III

CHROMATOGRAPHY OF PYRAZOLE DERIVATIVES ON ACETYLATED PAPER

	Substituent				*R_F values**	
	R_1	R_3	R_4	R_5	1	2
1	H	CH_3	H	CH_3	0.60	0.60
2	H	C_6H_5	H	C_6H_5	—	0.17
3	CH_3	CH_3	H	CH_3	0.70	0.90
4	C_6H_5	CH_3	H	CH_3	0.18	0.16
5	C_6H_5	H	H	Cl	—	0.24
6	C_6H_5	CH_3	H	Cl	0.18	0.07
7	C_6H_5	Cl	H	H	0.20	0.15
8	H	C_6H_5	H	NH_2	0.53	0.35
9	iso-C_3H_7	CH_3	H	NH_2	—	0.80
10	iso-C_3H_7	*p*-$C_6H_4NH_2$	H	NH_2	0.30	0.10
11	CH_3	*p*-$C_6H_4NH_2$	H	NH_2	0.30	0.20
12	C_6H_5	H	H	NH_2	0.26	0.16
13	C_6H_5	CH_3	H	NH_2	0.26	0.20
14	C_6H_5	H	NH_2	H	0.45	0.15
15	C_6H_5	NH_2	H	H	0.21	0.18
16	CH_2-C_6H_5	CH_3	H	NH_2	0.70	0.76
17	CH_3	CH_3	NO_2	CH_3	0.40	0.30
18	CH_3	CH_3	SCl	CH_3	0.50	0.33
19	iso-C_3H_7	CH_3	NO	CH_3	0.64	0.28
20	CH_2CH_2CN	C_6H_5	H	C_6H_5	—	0.23
21	C_6H_5	H	OCH_3	H	0.21	—
22	C_6H_5	H	HgCl	H	0.24	—
23	C_6H_5	H	HgBr	H	0.20	—
24	*p*-$NO_2 \cdot C_6H_4$	CH_3	NO_2	Cl	0.10	0.10
25	*p*-$SO_3H \cdot C_6H_4$	H	H	C_6H_5	0.20	0.17
26	NO**	CH_3	H	C_6H_5	0.30	0.22
27	$COCH=CHCO_2H$	CH_3	H	α-furyl	0.68	0.60
28	$COCH=CHCO_2H$	C_6H_5	H	α-furyl	0.20	0.20
29	iso-C_3H_7	CH_3	X***	OH	0.49	0.44
30	iso-C_3H_7	C_6H_5	X	OH	0.30	0.25
31	CH_2CH_2CN	CH_3	X	OH	0.00	0.15
32	$CH_2CH_2C_6H_5$	CH_3	X	OH	0.23	0.20
33	C_6H_5	CH_3	CH_2OH	Cl	0.19	—
34	C_6H_5	CH_3	H	Y***	0.63	0.79
35	C_6H_5	CH_3	H	Z***	0.56	0.40
36	C_6H_5	H	H	Y	—	0.30
37	C_6H_5	H	H	Z	—	0.20
38	C_6H_5	H	H	Y	—	0.60
39	C_6H_5	H	Y	H	—	0.59
40	C_6H_5	Z	H	H	—	0.10
41	C_6H_5	Y	H	H	—	0.60
42	C_6H_5	CH_3	H	Y	—	0.40

* Solvent systems: (1) chloroform (stationary phase)–80% ethanol; (2) chlorobenzene–80% ethanol.

** Pyrazoline ring.

*** X =  ;

Y = $-HN \cdot CO \cdot CH=C(OH) \cdot CH_3$;

Z = $-HN \cdot C(CH_3)=CH \cdot CO_2C_2H_5$.

TABLE IV

CHROMATOGRAPHY OF PYRAZOLE DERIVATIVES ON ACETYLATED PAPER

	Substituent				*R_F values**			
	R_1	R_3	R_4	R_5	*1*	*2*	*3*	*4*
1	C_6H_5	H	H	OH	0.57	0.40	0.60	—
2	C_6H_5	CH_3	H	OH	0.45	0.20	—	—
3	C_6H_5	H	OH	H	0.40	0.17	0.11	—
4	C_6H_5	OH	H	H	0.40	0.30	0.25	—
5	C_6H_5	OH	H	C_6H_5	0.40	—	—	—
6	C_6H_5	CH_3	H	SH	0.70	—	—	—
7	CH_3	CH_3	$COCH_3$	CH_3	—	0.68	—	—
8	CH_3	CH_3	COC_3H_7	CH_3	0.69	—	—	—
9	$CH_2 \cdot C_6H_5$	CH_3	COC_6H_5	CH_3	—	0.70	—	—
10	C_6H_5	CH_3	$COCH_3$	CH_3	0.50	0.30	—	—
11	C_6H_5	CH_3	COC_6H_5	CH_3	0.80	0.80	—	—
12	C_6H_5	H	$COCH_3$	Cl	—	0.24	—	—
13	C_6H_5	CH_3	$COCH_3$	Cl	0.20	0.20	0.20	—
14	C_6H_5	Cl	$COCH_3$	H	0.34	0.80	0.18	—
15	H	CH_3	$COCH_3$	CH_3	0.20	0.18	—	—
16	$COCH_3$	C_6H_5	H	C_6H_5	0.87	0.30	—	0.40
17	COC_6H_5	CH_3	H	CH_3	0.50	—	—	—
18	H	CH_3	H	COOH	—	0.30	0.50	0.40
19	H	C_6H_5	H	COOH	0.30	—	—	—
20	C_5H_{11}	C_4H_9	H	COOH	0.70	0.32	0.70	0.20
21	C_6H_5	H	H	COOH	0.40	0.20	0.36	0.30
22	C_6H_5	CH_3	H	COOH	0.54	0.36	0.50	0.45
23	C_6H_5	COOH	H	Cl	0.50	0.20	0.30	0.28
24	C_6H_5	CH_3	COOH	Cl	0.27	—	—	—
25	$CH_2 \cdot C_6H_5$	CH_3	H	COOH	0.70	—	—	—

* Solvent systems (stationary phase): (1) chloroform; (2) chlorobenzene; (3) isoamyl chloride; (4) benzyl chloride. Development solvent (in all cases): 80 % ethyl alcohol.

inductive effect of the electron-donor group CH_3, as the influence of this group on the tautomeric equilibrium between "oxo" and "oxi" forms[9] has already been demonstrated. The content of oxo-form in the equilibrium, already great for 1-phenyl-5-pyrazolone in $CHCl_3$, rises significantly with the introduction of a CH_3 group in position 3, and this considerably modifies the R_F. The NH_2 groups, on the contrary, always exist in amino form[10] and are not influenced significantly by a CH_3 group.

In the case of carboxylic acids and chloro-derivatives, which have a strong inductive $-I$ effect, we expect a rise in polarity on the introduction of a CH_3 group (positive $+I$ effect) in position 3, with a consequent modification of R_F.

For a substituent in position 1 or 3 we found an interesting rule: when substituting an alkyl (or benzyl) for a phenyl substituent, we observed a fall in R_F value in the solvent systems $CHCl_3$–80 % C_2H_5OH and C_6H_5Cl–80 % C_2H_5OH. As examples see Table III, No. 1 and 2, No. 3 and 4, No. 9, 13 and 16, No. 27 and 28, No. 29 and 30, and (weaker) Table IV, No. 22 and 25. So, if all the substituents on a pyrazole ring are the same, except one in position 1 or 3, the R_F values will show, with some degree of reliability, whether the substituent is an alkyl, aralkyl or a phenyl.

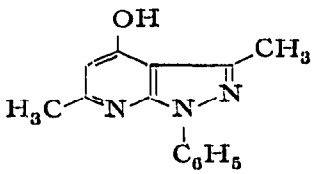
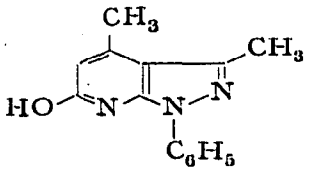
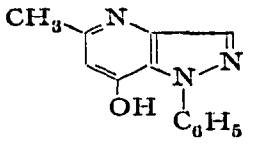
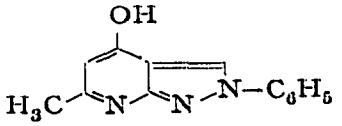
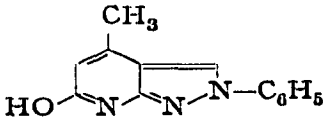
While investigating halogenated compounds that dissolve cellulose acetate as stationary phase solvents, and consequently solve acetylated paper, we came to

the conclusion that there are at least two groups of solvents that can be distinguished by the R_F of some of the pyrazole compounds. One group includes chlorobenzene and benzyl chloride, the other, chloroform and isopropyl chloride. The R_F of many substances is lower in the system chlorobenzene–80 % ethyl alcohol than in the system chloroform–80 % ethyl alcohol. This rule is valid for compounds having the most diverse functions and we could not explain it. We thought that the determining factor might be the dipole moment of the solvent, but we had to abandon this hypothesis because the μ for isopropyl chloride (1.93 in benzene) is nearer to the μ for benzyl chloride (1.85) than to the μ for chloroform (1.15 in benzene), and the difference between the μ for chlorobenzene (1.56) and benzyl chloride is greater than the difference between the μ for isopropyl chloride and benzyl chloride[11], thus invalidating the hypothesis.

Other alcohols (*n*- and iso-propanol, *n*- and *tert.*-butanol) were tried as development solvents, but the change in R_F of the compounds does not justify their use instead of ethyl alcohol.

TABLE V

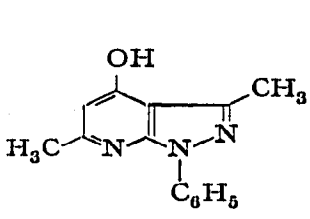
CHROMATOGRAPHY OF POLYCYCLIC COMPOUNDS OF PYRAZOLE WITH FUSED RINGS ON ACETYLATED PAPER

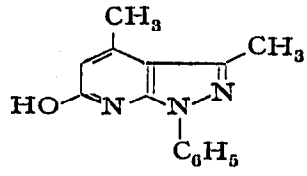
	Compound	R_F *values**	
		1	*2*
1		0.75	0.68
2		0.40	0.30
3		—	0.26
4		—	0.80
5		—	0.60

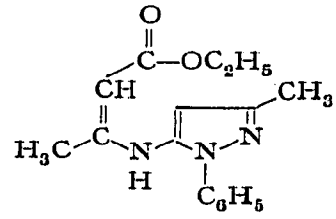
* Solvent systems: (1) chloroform–80 % ethanol; (2) benzyl chloride–80 % ethanol.

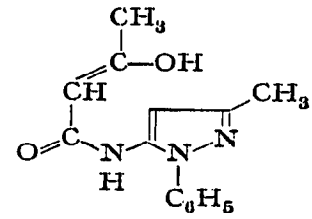
In the chromatography of ketones it is common practice to convert them to dinitrophenylhydrazine derivatives; as the dinitrophenylhydrazones are all coloured, there is no need for identification reagents. In our case (Table IV) the dinitrophenylhydrazones gave very long spots and an R_F value would have no meaning.

On the basis of our results we employed chromatography on acetylated paper to study the synthesis of polycyclic compounds with fused rings (Table V). Under different conditions[12] we attempted to synthesise the isomeric 1-phenyl-3,6-dimethyl-4-hydroxypyrazolo(3,4-*b*)pyridine (I) and 1-phenyl-3,4-dimethyl-6-hydroxypyrazolo-(3,4-*b*)-pyridine (II), from the intermediate compounds (III) and (IV), respectively.


(I)


(II)


(III)


(IV)

In contradiction to the opinion generally accepted after the work of BÜLOW[13], we found that the reaction of 1-phenyl-3-methyl-5-aminopyrazole with acetoacetic ester in boiling acetic acid gives compound (II), and not even traces of (I) were found. Both intermediate products (III) and (IV), in boiling acetic acid, always gave the same pyrazolo(3,4-*b*)pyridine (II). Compound (III) is probably converted to (IV) and from this the pyridine ring is formed in the usual way, to give (II). To confirm this hypothesis we boiled 1 g (III) for 15 min in glacial acetic acid and precipitated the compounds formed with water (all were very insoluble, with the exception of the 1-phenyl-3-methyl-5-aminopyrazole). A sample of the mixed solids dissolved in methanol and chromatographed showed the presence of 3 compounds: a little of the original compound (III), the pyrazolo(3,4-*b*)pyridine (II) and traces of the 1-phenyl-3-methyl-5-aminopyrazole. The R_F values of all of these compounds are sufficiently different from each other and from the R_F of (I), in the solvent system $CHCl_3$–80% C_2H_5OH, to leave no doubt about their identity (see Table V, No. 1 and 2; Table III, No. 13, 34 and 35). This proves that under these conditions compound (I) is not formed; in the first stage, probably compound (IV) is formed, but it could not be detected because the cyclisation to (II) is very fast.

ACKNOWLEDGEMENT

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SUMMARY

The chromatography of pyrazoles on acetylated paper is described. An attempt is made to correlate some of the chromatographic data with the structure of compounds. The influence of the inductive effect of the substituents is clearly seen, especially for 1-phenyl-3-methyl-5-x-pyrazoles. The R_F of an alkyl derivative (the

other substituents maintained) is greater than the R_F of the corresponding aryl substituted pyrazole.

In a condensed system, such as pyrazolo-pyridine, the α-pyridone can easily be distinguished from the γ-pyridone.

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