PAPER CHROMATOGRAPHY OF NITROFURAN DERIVATIVES

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(Received December 24th, 1965)

Paper chromatography is playing a rather important role in the analysis of drugs, because it can be used simultaneously for their identification, and for testing their purity and physiological activity (in the cases of isomeric forms with differences in physiological activity).

We carried out investigations on the paper chromatography of a number of nitrofuran derivatives, synthesized by ZHELYASKOV AND ZIKOLOVA[1], which have shown a high antibacterial, fungicidal and antiprotozoal activity[2,3].

The compounds under investigation are Schiff bases of 5-nitrofurfural with a series of aromatic amines. Compounds were chosen which, besides a high biological activity, showed related chemical structure and isomeric forms. The latter was done in order to find conditions for carrying out chromatography and solvent systems with the best separation for this group of compounds. In addition we also included three well known nitrofuran derivatives: 5-nitro-2-furaldehyde semicarbazone (furacin, nitrofurazone), N-(5-nitro-2-furfurylidene)-1-aminohydantoin (nitrofurantoin, furadantin) and N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone (furoxone, furazolidone).

Some valuable data are given in the literature on the paper chromatography of furacin, its determination in foods[6] and the detection of its decomposition products[4]. A communication has also appeared more recently on the paper chromatographic separation of furacin, furadantin and furoxone[5]. Use is chiefly made of hydrophilic solvent systems such as *n*-butyl alcohol–acetic acid–water (4:1:5)[4,5]; *n*-butyl alcohol–ammonia 10%–water (12:1:7)[5]; a less hydrophilic system, chloroform–isopropyl alcohol–potassium benzoate[6]; and from amongst the anhydrous systems, the system dimethylformamide/dioxan–methanol (9:1)[5] was examined without success. U.V. light was used for visualisation of the spots.

The present paper includes results from chromatographic studies on nitrofuran derivatives in nine solvent systems (Table I), selected with regard to the acid, neutral and basic characteristics of the compounds investigated and to their rather differing solubility and polarity. For the more lipophilic compounds, the systems S_3, S_4, S_5 and S_6 from Table I were examined as they were shown to be best fitted for compounds of this type[7]. We have also used systems with a stationary phase of dimethylformamide. Each of the systems under investigation has shown some advantages or disadvantages with respect to single compounds. But, in general, we arrived at the conclusion, that the anhydrous systems with a stationary phase of dimethyl-

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TABLE I

SOLVENTS

No.	*Immobile phase*	*Mobile phase*	*Time of chromatography (h)*
S_1	—	*n*-Butanol–acetic acid–water (4:1:5)	11
S_2	—	*n*-Butanol–2.5% NH_3 (1:1)	11
S_3	—	Butyl acetate–H_2O (1:1)	3
S_4	—	Tetrachloromethane–acetic acid (50:1)	$5^1/_2$
S_5	—	Benzene–methanol–H_2O (2:1:1)	3
S_6	—	Petroleum ether (b.p. 60–70°)–methanol–water (2:1:1)	3
S_7	Dimethylformamide	Chloroform	2
S_8	Dimethylformamide	Benzene	2
S_9	Dimethylformamide	Benzene–cyclohexane (4:1)	2

formamide and a mobile phase of chloroform, benzene or, better still, benzene–cyclohexane (4:1) are best fitted for identifying and separating of compounds from this group, as well as for detecting admixtures and products of decomposition. We have established that this also refers to furacin, furadantin and furoxone, which are more satisfactorily, reliably and quickly identified and examined for purity in the system dimethylformamide/benzene–cyclohexane (4:1) than in all the other systems applied up to now. The spots are visualized not only by observation in U.V. light, but also by spraying them with a solution of *p*-dimethylaminobenzaldehyde, after which yellow, orange or red spots are obtained.

EXPERIMENTAL

Materials

The Schiff bases of 5-nitrofurfural, given in Table II, were synthesized by ZHELYASKOV AND ZIKOLOVA[1], and characterised by their m.p. and nitrogen content. Furacin, furadantin and furoxone were supplied by Boehringer, Mannheim.

The initial amines from which the Schiff bases were prepared, had p.a. purity; they were also used as reference substances.

The solvents necessary for preparing the solvent systems, described in Table I, were freshly distilled and mixed 24 h before use.

Chromatography

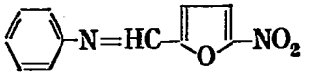
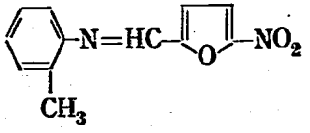
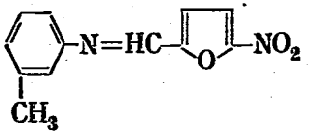
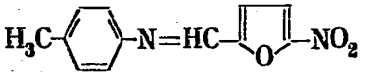
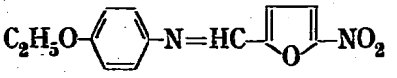
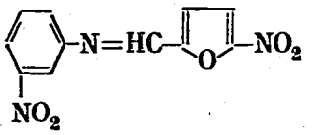
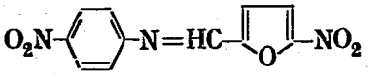
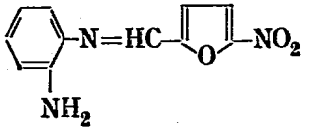
The substances examined were dissolved in alcohol by heating, at a concentration 1 mg/ml and 20–40 γ (0.02–0.04 ml) were applied to Schleicher & Schüll 2043 bMgl paper (13 × 38 cm). The development was carried out by the descending technique at a temperature of 20–22°. The paper strips with the samples were left to equilibrate with the systems S_1 to S_6 overnight in the chromatographic chambers, after which the mobile solvent was poured into the glass troughs. With solvent systems S_7–S_9, the paper strips, to which the samples had been applied, were im-

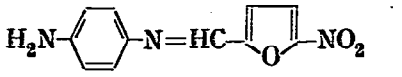
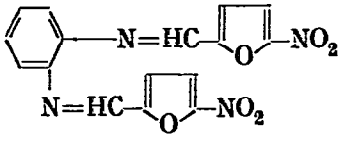
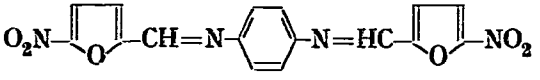
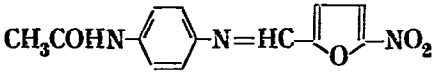
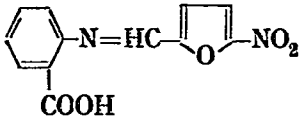
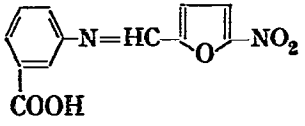
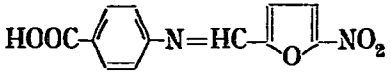
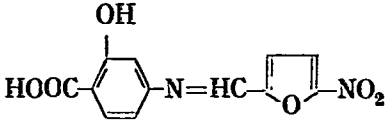
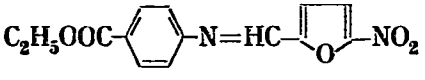
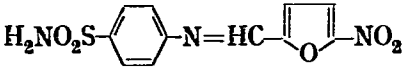
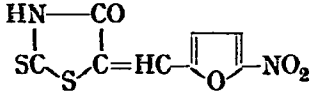
TABLE II

R_F VALUES OF THE INVESTIGATED SUBSTANCES

The R_F values are given for the center of the spots. The oblong spots are marked ↕, and the mark ↕ without a figure shows that the spot tails the full length of the chromatogram. The mark ↑ or ↓ indicates that the R_F value is given for the center of a spot, from which are tails either upwards or downwards, respectively.

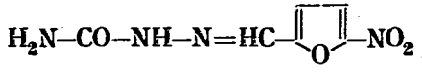
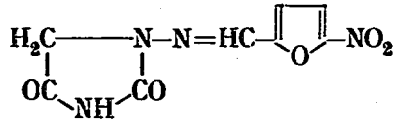
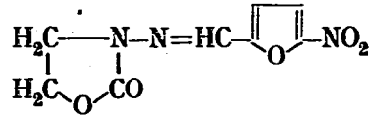
Fc = furacin; Fd = furadantin; Fs = furoxone.

No.	*Structural formula*	*Solvent systems*									*Colour of the spots*		
		S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	*Day-light*	*U.V.*	*1% p-Dimethylamino-benzaldehyde*
1		0.91	0.90	0.95	0.55↑	0.96	0.80	0.66	0.93	0.94	pale yellow	orange	pale yellow
2		0.90	0.91	0.92	0.61	0.97	0.77	0.65	0.90	0.93	pale yellow	pale brown	pale yellow
3		0.90	0.91	0.92	0.60	0.97	0.76	0.65	0.90	0.92	pale yellow	pale brown	pale yellow
4		0.90	0.91	0.92	0.60	0.97	0.76	0.65	0.90	0.92	pale yellow	pale brown	pale yellow
5		0.88	0.90	0.96	0.50↕	0.98	0.72	0.58	0.88	0.89	yellow	yellow	yellow
6		0.89	0.88	0.91	0.24↕	0.97	0.32↕	0.55	0.90	0.92	pale yellow	dark	yellow
7		0.89	0.87	0.94↓	0.00↑	↕	0.00	0.56	0.90	0.92	red	dark orange	red
8		0.90	0.78	↕	0.12↕	0.72↕	0.04	0.56	0.83	0.80	pale yellow	bright yellow	pale yellow

9		0.52	0.50↑↓	↑↓	0.00	↑↓	0.04	0.50	0.79	0.72	red	dark	red
10		0.90	0.84	0.00	0.67↑	0.78↑↓	0.02	0.61	0.87	0.87	pale yellow	yellow	—
11		0.50↑	0.00	0.00	0.00	0.78↑↓	0.04	↑↓	0.88	0.88	—	—	red
12		0.62	0.70↓	↑↓	0.00	0.05	0.02	0.53	0.81	0.73	pale yellow	—	orange
13		0.87	0.24	0.85	0.48↑↓	0.18	0.02	0.50	0.54	0.62	—	bright blue	pale yellow
14		0.79	0.10	0.52	0.02↑	0.05	0.00	0.22	0.18	0.20	—	green	lemon yellow
15		0.83	0.07	0.62↓	0.02↑	0.03	0.00	0.34	0.28	0.25	—	pale yellow	orange
16		0.84	0.10	0.55↑↓	0.00	0.00	0.00	0.04↑	0.05↑	0.05↑	pale yellow	pale yellow	yellow
17		0.91	0.91	0.92↓	0.30↑↓	0.99	0.60↑↓	0.50	0.89	0.91	—	—	yellow
18		0.64	0.43	0.26↑↓	0.00	0.00	0.00	0.22	0.08	0.06	—	yellowish green	yellow
19		0.90	0.63	0.92	0.28↑↓	0.65	0.00	0.43	0.67	0.64	yellow	dark	yellow

(continued on p. 450)

TABLE II (*continued*)

No.	*Structural formula*	*Solvent systems*									*Colour of the spots*		
		S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	*Day-light*	*U.V.*	*1% p-Dimethylamino-benzaldehyde*
Fc		0.70	0.56	0.00↑	0.00↑	0.00	0.00	0.32	0.40	0.48	pale yellow	pale yellow	—
Fd		0.58	0.13	0.00↑	0.00	0.00	0.00	0.43	0.55	0.65	pale yellow	pale yellow	—
Fs		0.72	0.58	0.00↑	0.00	0.00	0.00	0.52	0.65	0.72	pale yellow	pale yellow	—

pregnated with a 30 % methanolic solution of dimethylformamide, after which they were dried between two filter papers and allowed to air dry for 15 min, and equilibrate in the chambers for another half hour. When the front of the mobile solvent had moved nearly 28 cm from the start line, the chromatograms were withdrawn and left to dry in the air, after which the spots visible in day light were marked, then those visible in U.V. light and finally the paper was sprayed with a 1 % solution of *p*-dimethylaminobenzaldehyde in 8 % hydrochloric acid. The colours obtained and the R_F values, which represent the average values from 5 to 10 experiments, are given in Table II.

Detection of decomposition products of the compounds obtained as a result of the influence of water, acids and bases

Some aqueous solutions, with a concentration of 2 mg/ml, were prepared from the Schiff bases of 5-nitrofurfural and *o*-, *m*- and *p*-aminobenzoic acids and aminosalicylic acid, which in contrast to the other compounds insoluble in water (furacin, furadantin and furoxone are not taken into consideration) show a certain, though rather low solubility in water. At first the solutions were a light yellow colour which grew darker after 2–3 days. Those which had an insoluble residue were mixed with a little alcohol until a clear solution was obtained, before carrying out the chromatography. The latter was carried out according to the method described in system S_9, after three, seven and more days, and compared with fresh solutions of the compounds, and fresh and aged solutions of the amine.

The effect of acids and bases was followed by adding 0.05 ml 1 *N* HCl or KOH to 1 ml of the alcoholic solutions of the compounds (concentration 1 mg/ml), to give a solution approx. 0.05 *N* with respect to the acid or base. These solutions were left for half an hour at room temperature and refluxed on a steam bath for another half hour, after which they were applied to the paper, and chromatographed in the system S_9. Alcoholic solutions of the substances, as well as alcoholic solutions of the initial amines treated in the same way, were used as reference substances.

RESULTS AND DISCUSSION

Colour of the spots

Most of the compounds under investigation are coloured and appear on the chromatogram as yellow spots. Red is the typical colour of the *p*-nitroaniline and *p*-aminoaniline derivatives. In U.V. light, some of the compounds give bright fluorescent spots and others dark spots. The different fluorescence of the isomeric compounds (derivatives of *o*-, *m*-, and *p*-aminobenzoic acid and *o*- and *p*-phenylenediamine) and the colours obtained with *p*-dimethylaminobenzaldehyde are also characteristic. The spot appears as a bright blue fluorescence when the carboxylic group is in the *ortho* position, pale green when it is in the *meta* position and in the *para* position there is no fluorescence. On the other hand, the most intense colouring (orange) with *p*-dimethylaminobenzaldehyde appears with the *para* derivative. The *meta* derivative gives a lemon-yellow colour and the *ortho* a light yellow one.

When there is an amine group in the *ortho* position (*o*-phenylenediamine) the spot has a bright yellow fluorescence which diminishes if this amine group is condensed with another 5-nitrofurfural molecule. When the amine group appears in the *para* posi-

tion, the compound has a red colour in daylight, but in U.V. light appears dark. These differences thus make it possible to identify these isomers with certainty even in systems where their R_F values are rather close.

Furacin, furadantin and furoxone are pale yellow in daylight and in U.V. light. Unlike the other compounds examined they give no colour with *p*-dimethylaminobenzaldehyde. With an alkaline solution furacin forms a bright red spot, by which it may be differentiated from all the other nitrofuran derivatives investigated.

Shape of the spots

In most of the systems examined the spots are well formed, round or slightly oval. But a number of the compounds in the third, fourth, fifth and sixth solvent systems form an extended spot or remain at the start line. Obviously, in these systems the distribution equilibrium is reached rather slowly, and in some cases is not attained because the substances remain at the start line.

In system nine sharp round spots are obtained, and even compounds with small differences in R_F value are very well separated.

R_F values. Influence of functional groups

The simplest of all the compounds under investigation is 5-nitrofurfurylidene-aniline (therefore it will serve as comparative base for the chromatographic behaviour of all the other compounds). It is characterized, in general, by its high R_F values in all the systems utilized, which is an indication of its lipophility. Its R_F values are comparatively lower in systems S_4 and S_7, which contain tetrachloromethane and chloroform in the mobile phase. In general all the compounds under investigation showed lower R_F values in these two systems.

Introduction of a methyl group in the *ortho*, *meta* or *para* position of the benzene nucleus of 5-nitrofurfurylidene-aniline produces no changes in the chromatographic behaviour of the compounds. An ethoxy group in the *para* position causes a slight lowering of the R_F values, but in general the behaviour in the different solvent systems does not change. The introduction of a nitro group into the *meta* or *para* position also causes very little change in the R_F value in the systems, where well formed spots are obtained. Some changes are observed with the presence of an amine group in the benzene nucleus, particularly when it is in the *para* position (the R_F values are lower). There are characteristic differences between the *ortho* and *para* amino derivatives in the acid system S_1. The *para* amino derivative in this system has a low R_F value because of an increase in dissociation and hence an increase of the solubility in the immobile aqueous phase. This typical behaviour of the amine group is inhibited in *ortho* position. There is a similar effect in systems with a stationary phase of dimethylformamide. The higher R_F values of the *ortho* derivative, compared to those of the *para* derivative, may be explained by the reduced possibility of formation of intermolecular hydrogen bridges between the amine group in the *ortho* position and the immobile phase. Less soluble compounds are obtained by reacting the amine groups with a second 5-nitrofurfurylidene radical; these are difficult subjects for chromatography. By acetylating the amine group in the *para* position the behaviour in the acid system S_1 is mainly changed. The basic properties of the compounds are reduced.

The R_F values are shown to be dropping most sharply with the presence of a

carboxyl group in the benzene nucleus. This shows an increase of the solubility in the more polar immobile phase. Typical of the acid properties are the high R_F values in the acid systems S_1 and low R_F values in the alkaline system S_2. This is explained by inhibition of the dissociation in the first system, and by its potentiation in the second. The position of the carboxyl group is also of importance, the highest R_F values being shown by the compound with the carboxyl group in the *ortho* position, which may be explained by the formation of an intramolecular hydrogen bridge. The compounds possessing carboxyl groups in the *meta* and *para* positions have related R_F values, but are not identical. In the presence of both hydroxylic and carboxylic groups together, the increase of the polarity becomes still more apparent, especially in the systems with dimethylformamide. Obviously, the possibility for intermolecular hydrogen bridge formation with the immobile phase are still more enhanced. The typical behaviour of the carboxylic group vanishes after its esterification and the R_F values increase again.

The $-SO_2NH_2$ group strongly increases the polarity of the compound, especially in the systems with stationary dimethylformamide, where it gives lower R_F values than with the carboxylic group.

The rhodanine derivative (No. 19 in Table II) behaves as an acid, with a higher R_F value in the acid system S_1 and a lower one in the alkaline S_2. It behaves like furacin, furadantin and furoxone, which is due to the presence of a carbonyl group. In general their R_F values are lower, and in the systems S_3, S_4, S_5 and S_6 they do not move at all. In the dimethylformamide systems all three compounds show R_F differences large enough for separations.

Detection of admixtures and decomposition products

The nitrofuran derivatives under investigation, as Schiff bases, may contain admixtures of the amines from which they were prepared or from hydrolysis. The second component, 5-nitrofurfural, cannot be detected directly because of its volatility. That is why our attention was directed towards examining the amines, especially those which are non-volatile. The conversion of the volatile components into non-volatile ones would complicate their detection in admixtures and in fact was not a subject of our investigation.

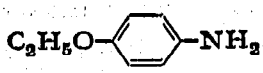
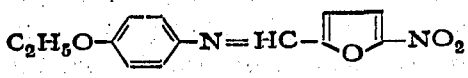
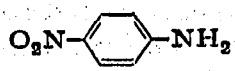
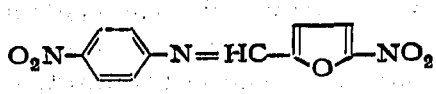
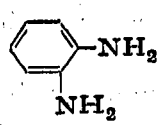
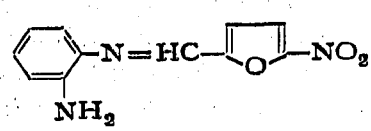
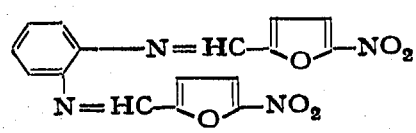
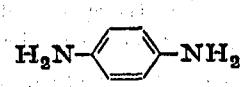
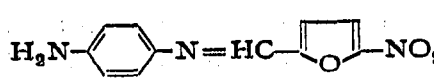
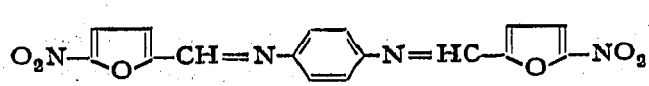
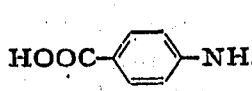
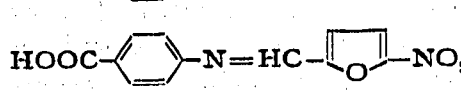
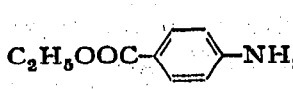
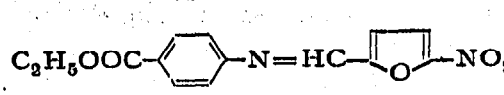
Most of the initial amines, chromatographed at the same time as reference compounds, have shown lower R_F values than the corresponding derivatives and have been detected more easily. It is of interest that the R_F values were changed differently by blocking the amine group of the various amines with 5-nitrofuraldehyde. In *ortho*-, *meta*- and *para*-aminobenzoic acids no changes were shown at all. It seems that the carboxylic group has a dominating influence upon the R_F values and veils the influence of the amine group. It is evident that the ΔR_M value in this case depends not only on the character of the functional group introduced, but also on the type and position of the other substituents in the benzene nucleus. Table III gives the ΔR_M values obtained as a result of the condensation of the amine group in the different aromatic amines used with 5-nitrofuraldehyde.

Stability of the compounds

The chromatograms of the alcoholic solutions in mg/ml concentration have shown no changes over a period of 5–6 days. The aqueous solutions and suspensions

TABLE III

R_F VALUES OF AROMATIC AMINES AND THE CORRESPONDING SCHIFF BASES WITH 5-NITROFURALDEHYDE IN THE SYSTEM DIMETHYLFORMAMIDE/BENZENE–CYCLOHEXANE (4:1)

Structural formula	R_F	R_M	ΔR_M
	0.75	−0.477	
	0.89	−0.908	−0.431
	0.75	−0.477	
	0.92	−1.061	−0.584
	0.44	+0.105	
	0.80	−0.602	−0.707
	0.87	−0.826	−0.224
	0.14	+0.788	
	0.72	−0.410	−1.198
	0.88	−0.865	−0.455
	0.25	+0.477	
	0.25	+0.477	0.0
	0.70	−0.368	
	0.91	−1.005	−0.637

of the *ortho*, *meta* and *para* derivatives of the aminobenzoic acids yielded two spots after 5–6 days (Fig. 1). One of these spots corresponds to the compounds alone, but the second spots have various differing R_F values and do not give a colour with *p*-dimethylaminobenzaldehyde. The second spots are not due to traces of the initial amines, because in these compounds all the initial amines have the same R_F values as their derivatives. They cannot be identified as nitrofurfural because, in such a case, all three compounds would have shown the same R_F value, whereas in this case all three are different. No amine group is present because they do not give a colour with *p*-dimethylaminobenzaldehyde. Probably, the carboxylic groups have also undergone some changes, because the R_F values sharply increase in the *meta* and *para* compounds. The examinations carried out in the microbiological laboratory of the institute have shown that these changes considerably reduce the biological activity of the preparations.

All nitrofurfurylidene derivatives decompose under the influence of 0.05 *N* acids and bases in alcoholic solution. The decomposition in acid medium is lower and the chromatogram shows two spots, one of which corresponds to the compound under investigation and the other to the amine obtained as a result of the decomposition. Decomposition proceeds faster in alkaline medium; the spot of the compound under investigation (especially after heating the solution) disappears and other spots appear on the chromatogram; some of these were not identifiable. Figs. 2 and 3 are given as

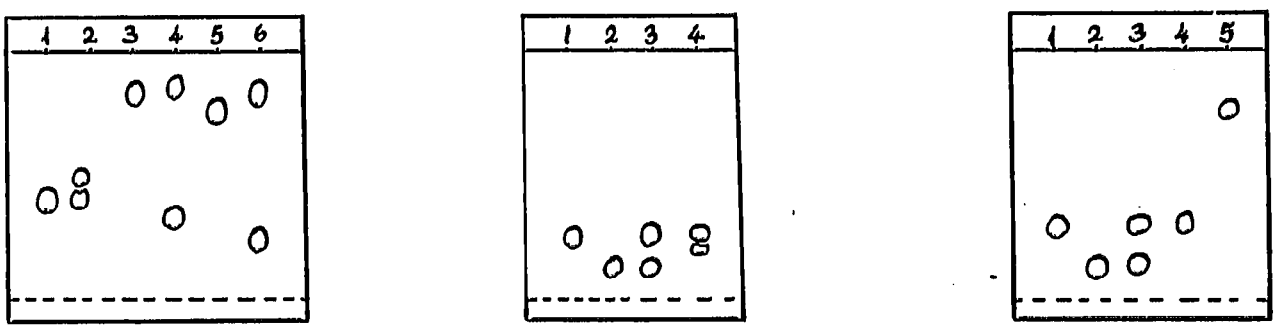


Fig. 1. System: dimethylformamide/benzene–cyclohexane (4:1). 1 = (5-nitrofurfurylidene)-*o*-aminobenzoic acid; 2 = the same compound after aging in an aqueous solution for 6 days; 3 = (5-nitrofurfurylidene)-*m*-aminobenzoic acid; 4 = the same compound after aging in an aqueous solution for 6 days; 5 = (5-nitrofurfurylidene)-*p*-aminobenzoic acid; 6 = the same compound after aging in an aqueous solution for 6 days.

Fig. 2. System: dimethylformamide/benzene–cyclohexane (4:1). 1 = *p*-phenetidine; 2 = (5-nitrofurfurylidene)-*p*-phenetidine; 3 = compound 2 in alcohol 0.05 *N* with respect to HCl, after heating for 30 min; 4 = compound 2 in alcohol, 0.05 *N* with respect to KOH, after heating for 30 min.

Fig. 3. System: dimethylformamide/benzene–cyclohexane (4:1). 1 = *p*-aminobenzoic acid ethyl ester; 2 = (5-nitrofurfurylidene)-*p*-aminobenzoic acid ethyl ester; 3 = compound 2 in alcohol, 0.05 *N* with respect to HCl, at room temperature after 30 min; 4 = compound 2 in alcohol, 0.05 *N* with respect to KOH, at room temperature after 30 min; 5 = compound 2 in alcohol, 0.05 *N* with respect to KOH, after heating for 30 min.

an illustration of the changes occurring with the phenetidine and anesthesine derivatives. The anesthesine derivative completely decomposes in alkaline medium to anesthesine and the latter is converted on heating to *p*-aminobenzoic acid (R_F 0.25).

ACKNOWLEDGEMENTS

The author is indebted to Prof. L. ZHELYASKO and Mrs. Sv. ZIKOLOVA for the gift of the compounds synthesized by them, as well as to Mrs. Dr. A. KOLUSHEVA for her great interest and the critical remarks on the work.

SUMMARY

Some investigations have been carried out on the paper chromatography of a group of new nitrofuran derivatives, along with three known preparations of the same group, *viz.* furacin, furadantin and furoxone. Nine solvent systems were employed, and it was established that for the identification and separation of this group of compounds, together with the detection of isomeric forms and admixtures, systems with a stationary dimethylformamide phase and a mobile phase of chloroform, benzene or better still benzene–cyclohexane (4:1) were best. The decomposition of the nitrofuraldehyde derivatives due to the influence of water, acids and bases was investigated. The influence of functional groups on the R_F values is discussed. Is was shown that the ΔR_M value obtained by blocking the aromatic amine group with 5-nitrofuraldehyde varies in each case and depends on the type and position of the other substituents in the benzene nucleus.

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