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Thin-Layer Chromatography of Imino-oxazolidinones: Separation, Identification and Estimation

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ABSTRACT

Imino-oxazolidinones, the cyclocondensation products of chloroacetanilides with potassium cyanate and their condensation products with vanillin including isomers have been chromatographed on starch bound silica gel thin-layers using one and two component solvent systems and effects of various properties of developing solvents and migrating species on R_F values have been investigated in addition to the separation, identification and estimation.

Key words: isomers, iminooxazolidinones, Thin layer chromatography.

INTRODUCTION

Although thin-layer chromatographic (TLC) studies on heterocyclic organic compounds including thiazolidinones[1-3] are well documented and numerous reports are also available on isomers but imino-oxazolidinones have not been investigated hitherto. This aroused our interest to carry out TLC studies, as this technique is superior to others in providing rapid and better separation, on a few imino-oxazolidinones including isomers. The effect of various properties of developing solvents and migrating species on R_F values and separation of isomers were investigated. The relationship between ë max and infrared spectral frequencies of characteristic group(s) and R_F values were used in the identification of compounds in their mixture(s) in resolving solvents.

EXPERIMENTAL

Materials

Imino-oxazolidinones, obtained by cyclocondensation of chloroacetinilides with potassium cyanate in dry ethanol followed by refluxing for ~ 2 h, washing of residues with water, and their condensation products obtained by their reaction with vanillin in dry ethanol in the presence of anhydrous sodium acetate followed by refluxing for 3-4 h, were purified by double crystallization

from acetone. The composition of each product was confirmed by elemental analysis, molecular weight determination (Rast method), and infrared, ^{1}H and ^{13}C NMR spectroscopy as reported elsewhere[3].

In synthetic work reagents grade chemicals (95-97%) were used as supplied where as in TLC studies analytical (AR)/ HPLC-grade solvents were used.

Procedures

Glass plates (20×20 cm) were coated by spreading an aqueous slurry containing homogeneous mixture of silica gel (BDH) and starch (19:1, w/w) with a laboratory- built applicator[4] and coated plates were air dried. Both sides of gel layer were scraped of to a width of 5 mm and the coated plates were activated by heating ca.80^{0}C for 1h before use.

In order to ensure the compactness of spots spotting of standard solution of samples in acetone was done on warm plates. For qualitative studies sample solutions were spotted as small drops using fine glass capillaries on 0.1 cm thick layer whereas in quantitative work known volumes of solutions were applied with a micropipette to a 0.2 cm thick gel layers. Sample solutions were applied as a series of spots or bands in a line 2 cm from the edge of the plate. Oven dried loaded plates were developed in rectangular glass chamber, pre-saturated with developing solvent to ensure reproducibility of results, with ground- in- lids by ascending technique. On development for ca. 8 cm the plates were removed from the chamber. Although all the spots were visualized in day light, some were more clearly discernible in iodine vapor.

For quantitative analysis chromatogram bands were scraped off, centrifuged with 1-2ml acetone three times and volume was made up to 5 ml. The absorbance of the solution of each mixture component was measured on SP-65 Uv-Vis spectrophotometer at the wave length of maximum absorption (ë max) of the solute and concentration was calculated from linear calibration curves obtained in the range of 0-400 µg under identical conditions of medium (acetone) and temperature (20±2^{0}C).

RESULTS AND DISCUSSION

Effects of various parameters on R_F values

The effect of gel layer thickness and presence of other compounds (mixture) on R_F values were examined for some isomeric oxazolidinones as migrating spots in resolving solvents. The R_F data for ternary and binary mixtures of isomers in Table 2 and 3 show a lowering of R_F values with increase in gel layer thickness whereas almost identical R_F values obtained when the compounds migrating individually (table 1) and in mixture (table2) results that the migration is independent of the presence of other compounds.

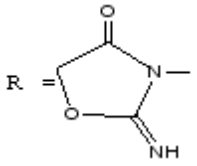
The effect of solvents polarity on R_F values was studied with all oxazolidinones in oxygen-containing solvents. The order of R_F values, BuOH < PrOH < EtOH MeOH corresponding to the solvent polarity, shows that R_F values and their sequence is governed by the solvent polarity. In non-oxygen-containing solvents order of R_F values $CHCl_3$ C_6H_6 petroleum ether n-hexane, being consistent with the sequence in their dielectric constants, supports the inference arrived on oxygen-containing solvents. The higher R_F values in aromatic solvent benzene than in aliphatic solvent containing same carbon atoms such as n- hexane clearly show that aromatic solvents are better than the aliphatic solvents in establishing R_F values because double bonds of benzene forms weak hydrogen bonds.

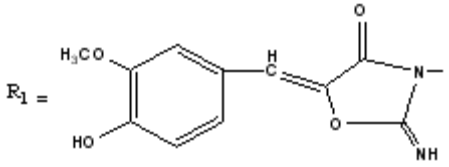
TLC studies of isomeric ternary nitro- and methoxy- oxazolidinone mixtures revealed particular R_F orders depending on the nature of the solvent, i.e. whether it is oxygen-containing or non-oxygen- containing or it is a one or two component system. In almost all the oxygen-containing solvents irrespective of the nature of functional groups and polarity of solvents, compounds of both the series exhibit R_F order p <; in mixture solvents the same order is established generally. This reveals that position of substituted group or steric hindrance in solutes mainly governs the R_F value irrespective to other properties of solutes and solvents.

To study the effect of nature of substituent on R_F values, p-substituted oxazolidinones were chosen as in this position the steric effects are

Table 1: Color, Rf , λ max and IR frequencies values of analysed compounds in various one and two solvent systems

Compound	Spot color RF 100	λmax values (cm -1) ^{v}HC=N	IR frequencies											
			MeOH	EtOH	PrOH	BuOH	Et2O	EtOAc	AcOH	Petroleum ether	n-Hexane	CHCl3	Benzene	
R-C_6H_4-NO_2(o)	Yellow green	360	1595	89	84	83	86	60	91	87	00	00	73	40
R-C_6H_4-NO_2(m)	olive	350	1591	88	83	80	72	61	90	86	00	00	35	39
R-C_6H_4-NO_2(p)	Yellow green	360	1595	88	82	79	85	65	89	85	00	00	45	31
R-C_6H_4-OCH_3(o)	Alice blue	340	1684	88	83	84	76	63	86	75	00	00	46	10
R-C_6H_4-OCH_3(m)	Yellow green	350	1665	87	82	82	69	63	84	73	00	00	22	00
R-C_6H_4-OCH_3(p)	Light yellow	345	1645	86	81	80	66	56	83	68	00	00	30	19
R1-C_6H_4-NO_2(o)	yellow	355	1598	87	82	83	74	70	90	76	00	00	33	21
R1-C_6H_4-NO_2(m)	Orange red	300	1593	84	81	81	77	64	88	74	00	00	24	24
R1-C_6H_4-NO_2(p)	yellowish	430	1632	80	79	74	89	62	83	73	00	00	36	22
R1-C_6H_4-OCH_3(o)	yellow	360	1664	90	82	81	67	65	85	75	00	00	28	10
R1-C_6H_4-CH_3(m)	Green yellow	330	1590	87	81	76	71	60	84	70	00	00	28	08
R1-C_6H_4-OCH_3(p)	brown	350	1595	86	80	74	80	58	82	68	00	00	32	00
Development time				25	42	45	30	15	12	10	10	10	10	15





RF 100						
n-hex-Ethyl acetate(v:v)				Benzene- Ethanol(v:v)		
2:1	1:1	1:2	4:1	3:2	1:1	2:3
34	66	87	82	86	95	92
31	62	85	81	85	85	90
23	61	86	78	86	94	89
21	59	79	63	87	92	89
30	62	80	57	88	93	90
28	50	77	55	88	91	89
32	60	85	84	86	96	92
33	53	78	78	85	92	94
29	59	80	78	86	90	95
30	45	77	79	87	96	88
29	77	70	41	88	90	87
23	48	71	38	87	79	87
25	20	17	36	27	26	32

Table 2: Qualitative resolution of isomeric mixtures

No	Mixture of isomeric oxazolidinones	Resolving solvent
1	R-C_6H_4-NO_2(o) or R-C_6H_4-NO_2(p) + R-C_6H_4-NO_2(m)(86) (85) (72)	Bu OH
2	R-C_6H_4-OCH_3(m) + R-C_6H_4-OCH_3(p) (62) (50)	n-hexane-EtOAs (1:1,v/v)
3	R_1-C_6H_4-NO_2(o) or R_1-C_6H_4-NO_2(m) + R_1-C_6H_4-NO_2(p) (74) (77) (89)	Bu OH
4	R_1-C_6H_4-OCH_3(o) + R_1-C_6H_4-OCH_3(m) or R_1-C_6H_4-OCH_3(p) (79) (41) (38)	Benzene –EtOH (4:1,v/v)
5	R-C_6H_4-NO_2(o) + R-C_6H_4-NO_2(m) + R-C_6H_4-NO_2(p) (73) (35) (45)	$CHCl_3$
6	R-C_6H_4-OCH_3(o) + R-C_6H_4-OCH_3(m) + R-C_6H_4-OCH_3(p) (46) (22) (30)	$CHCl_3$

Values in parenthesis are R_fx100

smaller than in the other positions and in such cases the nature of substituent group predominates. From the higher R_F values of nitro substituted oxazolidinones than the methoxy compounds, the effect of electron withdrawing nature of the substituted groups (NO_2 OCH_3) is evident.

The effect of functional groups of solvent on R_F values has been studied in MeOH and MeCOOH. The higher R_F values of all the compounds in MeOH than in MeCOOH clearly reveal better effect of alcoholic group on R_F values than carboxylic group. In two component mixture solvents of n–hexane–EtOAc (2:1,1:1 and 1:2,v/v) ratio R_F values of all the migrating compounds are intermediate to the R_F values obtained in pure solvents as expected but benzene –EtOH mixture solvents (3:2,1:1and 2:3,v/v) showed abnormal R_F values (higher than pure solvents). The exceptionally higher values in benzene –EtOH (3:2, 1:1 and 2:3, v/v) mixtures could be attributed to the formation of hemiaketals or ketals by deprotonated ethanol in the presence of benzene inert medium with ketonic group(s) of the oxazolidinones. However in benzene–EtOH (4:1, v/v) the R_F values lying in between pure solvents values could most probably be due to inadequate quantity of EtOH as compared to benzene to form the hemiketals or ketals.

Separation, identification and determination of oxazolidinones in mixture

Among different solvents tried for separation of oxazolidinones, chloroform showed the highest resolving capacity as it could resolve several mixtures of six compounds; the best resolution of diverse mixtures of three compounds, however, could only be achieved in benzene, n-hexane–EtoAc (1:1,v/v) and benzene–EtOH (4:1,v/v). The ternary and binary mixtures of isomeric compounds of both series (R and R_1) have also been resolved only by these solvents (Table 2).

The IR stretching frequency of the C=NH, a common characteristic group, and ë max of the isomeric oxazolidinones have been correlated with the R_F values in their resolving solvents. In R-C_6H_4-NO_2 isomers R_F order, meta para ortho is similar to that of the ë max and C=NH stretching values whereas in R-C_6H_4-OCH_3 isomers the R_F values fall in opposite order to that of their ë max values. In binary mixtures of R_1-C_6H_4-NO_2 and R_1-C_6H_4-OCH_3 isomers the R_F and ë max values as well as the C=NH frequencies are in identical orders. These R_F and spectral correlations were used for the identification of mixture components after separation.

Table 3: Quantitative analysis of isomeric oxazolidinones

Isomeric oxazolidinones in mixture	Amount loaded (µg)	Amount recovered (µg)	Mean	SD*	RSD**	Error (%)	R_F x 100	Resolving solvent
Experiment.1[a]								
R-C_6H_4-NO_2(o)	200	198.8, 199.4, 200	199.4	0.63	0.31	0.31	70	$CHCl_3$
R-C_6H_4-NO_2(m)	250	248.3, 249.6, 249.2	249.1	0.64	0.26	0.39	32	
R-C_6H_4-NO_2(p)	250	247.7, 245, 244.95	245. 9	1.5	0.65	1.64	42	
R-C_6H_4-OCH_3(o)	200	200, 196.9, 197.5	198.1	1.65	0.83	0.94	43	$CHCl_3$
R-C_6H_4-OCH_3(m)	250	250, 238.1, 244.4	244.2	5.94	2.43	2.3	20	
R-C_6H_4-OCH_3(p)	200	199.6, 198.3, 194.2	197.4	2.84	1.44	1.3	28	
R_1-C_6H_4-NO_2(o)	200	39.92, 39.5, 38.58	196.7	3.47	1.8	1.7	71	BuOH
R_1-C_6H_4-NO_2(p)	250	249.5, 243.6, 248.2	247.1	3.1	1.3	1.2	85	
R_1-C_6H_4-OCH_3(o)	200	198.9, 199.5, 196.2	198.2	1.78	0.90	0.93	78	Benzene –EtOH(4:1,v/v)
R_1-C_6H_4-OCH3(m)	250	249.4, 243.1,244.2	245.6	3.3	1.4	1.8	39	
R_1-C_6H_4-OCH_3(o)	200	198.9, 199.5, 196.2	198.2	1.78	0.90	0.93	78	Benzene –EtOH(4:1,v/v)
R_1-C_6H_4-OCH_3(p)	250	249.4, 243.1, 244.2	247.6	1.9	0.79	0.96	35	
Experiment 2[a]								
R-C_6H_4-NO_2(o)	150	148.1, 148.7, 150	148.9	0.95	0.64	0.69	70	$CHCl_3$
R-C_6H_4-NO_2(m)	250	250,248.75,249.27	249.3	0.64	0.26	0.28	32	
R-C_6H_4-NO_2(p)	200	195, 199.54, 195	196.5	2.62	1.33	1.7	42	
R-C_6H_4-OCH_3(o)	150	150, 147.5, 148.13	148.5	1.30	0.88	0.94	43	$CHCl_3$
R-C_6H_4-OCH_3(m)	250	248.8, 244.4, 244.375	245.8	2.53	1.03	1.7	20	
R-C_6H_4-OCH_3(p)	150	150, 148.3, 147.97	148.8	1.10	0.74	0.8	28	
R_1-C_6H_4-NO_2(o)	150	150, 148.8, 147.1	148.6	1.46	0.99	0.93	71	BuOH
R_1-C_6H_4-NO_2(p)	200	198.2, 192.7, 193.2	194.7	3	1.6	2.7	85	
R_1-C_6H_4-OCH_3(o)	150	148.9, 148.9, 143.3	147	3.2	2.2	2	78	Benzene-EtOH(4:1, v/v)
R_1-C_6H_4-OCH3(m)	250	246.9, 245.6, 245	245.8	0.95	0.39	1.7	39	
R_1-C_6H_4-OCH_3(o)	150	148.9, 148.9, 143.3	147	3.2	2.2	2	78	Benzene-EtOH(4:1, v/v)
R_1-C_6H_4-OCH_3(p)	250	246.9, 245, 245.6	248. 9	3.1	3.1	0.44	35	

SD*standard deviation RSD** relative standard deviation

Table 3. cont.

Experiment.3[a]								
Isomeric oxazolidinones in mixture	**Amount loaded (µg)**	**Amount recovered (µg)**	**Mean**	**SD***	**RSD****	**Error (%)**	**R_F x 100**	**Resolving solvent**
R-C_6H_4-NO_2(o)	100	98.125, 99.4, 98.75	98.75	0.625	0.63	1.25	70	$CHCl_3$
R-C_6H_4-NO_2(m)	250	248.8, 249.5, 247.9	248.8	0.83	0.34	0.5	32	
R-C_6H_4-NO_2(p)	150	149.5, 148.6, 148.2	148.8	0.69	0.47	0.81	42	
R-C_6H_4-OCH_3(o)	100	100, 98.75, 98.125	98.96	0.95	0.96	1.04	43	$CHCl_3$
R-C_6H_4-OCH_3(m)	250	249.4, 238.1, 248.1	245.2	6.2	2.5	1.9	20	
R-C_6H_4-OCH_3(p)	100	99.2, 98.3, 94.2	97.2	2.7	2.8	2.8	28	
R_1-C_6H_4-NO_2(o)	100	99.6, 96.7, 97.5	97.9	1.50	1.5	2.1	71	BuOH
R_1-C_6H_4-NO_2(p)	150	148.2, 148.2, 144.1	146.8	2.4	1.6	2.1	85	
R_1-C_6H_4-OCH_3(o)	100	98.9, 99.4, 98.3	98.9	0.56	0.56	1.1	78	Benzene-EtOH(4:1, v/v)
R_1-C_6H_4-OCH3(m)	200	200, 193.8, 198.8	197.5	3.30	1.7	1.3	39	
R_1-C_6H_4-OCH_3(o)	100	98.9, 99.4, 98.3	98.9	0.56	0.56	1.1	78	Benzene-EtOH(4:1, v/v)
R_1-C_6H_4-OCH_3(p)	200	200, 193.8, 198.8	197.96	2.57	2.57	1	35	

SD*standard deviation RSD** relative standard deviation

In order to test the application of the TLC method in the analysis of oxazolidinones various mixtures of isomeric compounds were analysed qualitatively (Table 2) on 0.1cm and quantitatively on 0.2cm thick layer (Table 3). The maximum amounts of isomers resolved from their mixture reveal the maximum separation limit of this method. All the results are reproducible.

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