

Short Communication

Thin-layer chromatography of thiazolidinones. I

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ABSTRACT

1,4-Thiazolidinones, the cyclocondensation products of ketoanils with thioglycolic acid, 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 beside the separation, identification and estimation.

INTRODUCTION

Although thin-layer chromatographic (TLC) and paper chromatographic studies on heterocyclic organic compounds are well documented and numerous reports are also available on isomers, 1,4-thiazolidinones have not previously been investigated. This led us to carry out TLC studies, as this technique is superior to others in providing rapid and better separation, on a few 1,4-thiazolidinones including their isomers. The effects of various properties of developing solvents and thiazolidinones on R_F values and the separation of isomers were investigated. The relationship between the infrared spectral frequencies of characteristic groups and R_F values in different solvents were used for the identification of compounds.

EXPERIMENTAL

Materials

1,4-Thiazolidinones were obtained by reaction of ketoanils [1] with thioglycolic acid in dry benzene followed by refluxing for 15–20 h and neutralizing the reaction mixture with sodium hydrogencarbonate. The composition of each product, purified by column chromatography using silica gel G as sorbent and acetone as solvent, was confirmed by elemental analysis and infrared spectrometry, as reported elsewhere [2].

In synthetic work reagent-grade chemicals were used as received whereas in TLC studies they were used after purification involving drying and distillation.

Procedure

For coating the plates an aqueous slurry containing a homogeneous mixture of silica gel G (BDH) and starch (19:1, w/w) was spread on glass plates (18 × 10 cm) with a laboratory-built applicator [3]. The coated plates were dried in air. Both sides of the gel layer were scraped off to a width of about 5 mm. Before use the coated plates were activated by heating at *ca.* 100°C for 1 h.

For qualitative studies warm plates (with a 0.10-cm thick layer), to ensure compactness of spots, were spotted with standard solutions of samples in acetone as small drops using glass capillaries whereas in quantitative work known volumes of solutions were applied with a micropipette to a 0.15-cm thick gel layer. Sample solutions were applied as series of spots or bands in a line 2 cm from the edge of the plate. The oven-dried loaded plates were developed in rectangular glass chambers with ground-in-lids by the ascending technique. To obtain reproducible results the development chambers were saturated with solvent before use. When development had proceeded for *ca.* 8 cm the plate was removed from the chamber. Owing to the dark colours produced by the analytes their spots were readily discernible in daylight.

For quantification the component bands were scraped off, treated with 15–20 ml of acetone to extract the thiazolidinones and the solutions were evaporated to 5 ml. The absorbances of the solutions were measured on a Bausch and Lomb Spectronic-20 spectrophotometer at the wavelengths of maximum absorption of the solutes and the concentrations were calculated from linear calibration graphs obtained in the range 0–500 µg under identical conditions of medium (acetone) and temperature (27 ± 2°C).

RESULTS AND DISCUSSION

Effects of various parameters on R_F values

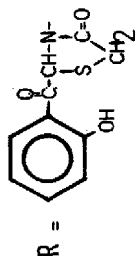
The effects of the development rate, gel layer thickness and the presence of other compounds on the R_F values were examined for some thiazolidinones as migrating spots in selected solvents. The R_F values determined at two development rates (measured at plate angles of 80° and 50° from the horizontal plane) are given in Table I. As can be seen, the R_F values were independent of development rate. Almost identical R_F values obtained when the compounds migrated individually (Table II) and in mixtures (containing up to six compounds); hence the migration is independent of the presence of other compounds. The R_F data for ternary and binary mixtures of isomers in Tables II and III show a lowering of R_F values with increase in gel layer thickness.

The effect of solvent polarity on R_F values was studied with all the thiazolidinones in both oxygen-containing and non-oxygen-containing solvents. The R_F orders, isobutanol < *n*-butanol, *n*-butanol < ethanol and carbon tetrachloride < dichloromethane < chloroform, corresponding to the solvent polarities, show that the R_F values and their sequences are governed by the solvent polarity.

TLC studies of isomeric ternary nitro- and methoxythiazolidinone mixtures and binary naphthyl compound mixtures revealed particular R_F orders depending on the nature of the solvent, *i.e.*, whether oxygen-containing or non-oxygen-containing, alcoholic or ketonic, or a one- or two-component system.

To study the effect of the nature of substituents on R_F values, *para*-substituted

TABLE I
EFFECT OF DEVELOPMENT RATE ON R_f VALUES



Developing solvent	Development rates (mm/h) ^a	$R_f \times 100^a$					
		RC_6H_5	RC_6H_4SH-o	$RC_6H_4NO_2-o$	$RC_6H_4NO_2-m$	$RC_6H_4NO_2-p$	
Acetone	69(43)	94(94)	93(93)	92(92)	89(90)	92(92)	
n-Butanol	45(30)	94(94)	95(94)	94(94)	87(87)	90(90)	
Chloroform	107(67)	00(00)	63(63)	43(43)	53(53)	63(63)	
Dioxane	71(45)	93(92)	98(98)	99(98)	94(94)	97(97)	
Diethyl ether	156(98)	00(00)	96(96)	86(86)	62(62)	62(62)	
Benzene-ethanol (7:3, v/v)	107(70)	70(70)	93(93)	78(78)	72(72)	65(65)	
Benzene-ethanol (2:3, v/v)	86(54)	97(98)	98(99)	94(93)	93(93)	98(97)	

^a First and second (in parentheses) values were measured at plate angles of 80° and 50° respectively.

TABLE II
SPOT COLOURS, SPECTRAL PARAMETERS AND $R_f \times 100$ VALUES FOR THIAZOLIDINONES

Compound	Spot colour	IR frequencies (cm^{-1})		$R_f \times 100$	Diethyl ether	Isobutanol	Ethyl acetate	Benzene	Toluene	Chloroform	Benzene-ethanol	
		$\nu \text{C-N}$	$\nu \text{C=O}$ (ring)								7:3 (v/v)	24:1 (v/v)
RC_6H_5	Light brown	1590	1720	00	80	94	00	00	00	00	70	87
$\text{RC}_6\text{H}_4\text{SH-}o$	Light brown	1600	1690	96	95	85	84	00	00	— ^a	90	100
$\text{RC}_6\text{H}_4\text{N-}o$	Dark brown	1590	1695	00	00	00	00	00	00	00	—	—
$\text{RC}_6\text{H}_4\text{NO}_2-o$	Light yellow	1600	1780	86	92	75	35	25	43	53	78	100
$\text{RC}_6\text{H}_4\text{NO}_2-m$	Yellow	1600	1740	62	85	70	19	14	53	63	72	100
$\text{RC}_6\text{H}_4\text{NO}_2-p$	Yellow	1600	1720	62	85	72	19	8	63	63	65	100
$\text{RC}_6\text{H}_4\text{OCH}_3-o$	Light yellow	1585	1780	00	92	82	00	00	00	00	91	100
$\text{RC}_6\text{H}_4\text{OCH}_3-m$	Brownish yellow	1585	1775	00	92	00	00	00	00	00	84	100
$\text{RC}_6\text{H}_4\text{OCH}_3-p$	Brownish yellow	1580	1760	00	89	84	67	1	00	00	89	100
$\text{RC}_{10}\text{H}_9-\alpha$	Light brown	1630	1800	00	85	97	50	00	00	00	100	100
$\text{RC}_{10}\text{H}_9-\beta$	Red-brown	1600	1720	00	85	95	50	46	00	00	93	87
$\text{RC}_6\text{H}_4\text{Cl-}p$	Yellowish brown	1580	1770	00	89	86	67	00	00	00	98	100
$\text{RC}_6\text{H}_4\text{Br-}p$	Brownish yellow	1585	1680	00	89	85	67	00	00	00	95	100
$\text{RC}_6\text{H}_4\text{I-}p$	Brown	1590	1780	00	89	85	67	00	00	00	96	100
$\text{RC}_6\text{H}_4\text{N}(\text{C}_2\text{H}_5)_2-p$	Purple-brown	1590	1690	00	00	00	00	00	00	00	—	90
$\text{RC}_6\text{H}_4\text{CH}_3-p$	Yellowish brown	1590	1690	98	78	94	67	00	00	00	75	92
$\text{RC}_6\text{H}_4\text{R-}p$	Yellowish brown	1580	1680	00	00	00	00	00	00	00	80	85
$\text{RC}_6\text{H}_4\text{C}_6\text{H}_4\text{R-}p$	Light brown	1575	1760	—	00	79	67	00	00	00	82	79

^a Dashes denote spreading of spots.

TABLE III
QUANTITATIVE ANALYSIS OF ISOMERIC THIAZOLIDINONE MIXTURES

Isomeric thiazolidinones in mixture	Experiment 1 ^a					
	Amount loaded (μg)	Amount recovered (μg)	M.D. (μg)	S.D. (μg)	R.S.D. (%)	Error (%)
RC ₆ H ₄ NO ₂ - <i>o</i>	150	148,149,148.5	0.33	0.41	0.28	1.00
RC ₆ H ₄ NO ₂ - <i>m</i>	150	149,149.5,150	0.33	0.41	0.27	0.33
RC ₆ H ₄ NO ₂ - <i>p</i>	150	150.5,148.5,149	0.78	0.85	0.57	0.45
RC ₆ H ₄ OCH ₃ - <i>o</i>	100	99,99.5,100.5	0.55	0.62	0.63	0.34
RC ₆ H ₄ OCH ₃ - <i>m</i>	100	100,99.5,99.5	0.22	0.24	0.24	0.34
RC ₆ H ₄ OCH ₃ - <i>m</i>	100	99.5,99,99	0.22	0.24	0.23	0.83
RC ₆ H ₄ OCH ₃ - <i>p</i>	100	99.5,98.5,99.5	0.44	0.47	0.48	0.83
RC ₁₀ H ₉ - <i>α</i>	100	99,99,99	0.00	0.00	0.00	1.00
RC ₁₀ H ₉ - <i>β</i>	60	59.5,59.5,58.5	0.44	0.47	0.80	1.39

Isomeric thiazolidinones in mixture	Experiment 2 ^a					
	Amount loaded (μg)	Amount recovered (μg)	M.D. (μg)	S.D. (μg)	R.S.D. (%)	Error (%)
RC ₆ H ₄ NO ₂ - <i>o</i>	150	148.5,149.5,148.5	0.44	0.47	0.32	0.78
RC ₆ H ₄ NO ₂ - <i>m</i>	300	298,298.5,298	0.12	0.23	0.08	0.61
RC ₆ H ₄ NO ₂ - <i>p</i>	300	299,299.5,300	0.33	0.41	0.14	0.17
RC ₆ H ₄ OCH ₃ - <i>o</i>	100	99.5,99.5,99.5	0.00	0.00	0.00	0.50
RC ₆ H ₄ OCH ₃ - <i>m</i>	60	60.5,59,59.5	0.55	0.62	1.04	0.57
RC ₆ H ₄ OCH ₃ - <i>m</i>	120	119.5,120,119	0.33	0.41	0.34	0.42
RC ₆ H ₄ OCH ₃ - <i>p</i>	60	59,59,59.5	0.12	0.23	0.40	1.39
RC ₁₀ H ₉ - <i>α</i>	60	59,60,59	0.44	0.47	0.79	1.12
RC ₁₀ H ₉ - <i>β</i>	100	99,98.5,99.5	0.33	0.41	0.41	1.00

Isomeric thiazolidinones in mixture	Experiment 3 ^a						<i>R_F</i> × 100	Resolving solvent
	Amount loaded (μg)	Amount recovered (μg)	M.D. (μg)	S.D. (μg)	R.S.D. (%)	Error (%)		
RC ₆ H ₄ NO ₂ - <i>o</i>	—	—	—	—	—	—	65	Benzene-ethanol (24:1, v/v)
RC ₆ H ₄ NO ₂ - <i>m</i>	—	—	—	—	—	—	49	
RC ₆ H ₄ NO ₂ - <i>p</i>	—	—	—	—	—	—	29	
RC ₆ H ₄ OCH ₃ - <i>o</i>	—	—	—	—	—	—	80	Ethyl acetate
RC ₆ H ₄ OCH ₃ - <i>m</i>	—	—	—	—	—	—	00	
RC ₆ H ₄ OCH ₃ - <i>m</i>	160	159,158.5,158.5	0.12	0.23	0.15	0.83	00	
RC ₆ H ₄ OCH ₃ - <i>p</i>	120	119,119.5,118.5	0.33	0.41	0.34	0.83	82	Toluene
RC ₁₀ H ₉ - <i>α</i>	—	—	—	—	—	—	00	
RC ₁₀ H ₉ - <i>β</i>	—	—	—	—	—	—	45	

^a M.D. = Mean or average deviation; S.D. = standard deviation; R.S.D. = relative standard deviation.

thiazolidinones were chosen, as in this position steric effects are smaller than in other positions and the nature of the substituent group predominates. The R_F values of substituted compounds were compared with that of unsubstituted phenylthiazolidinone. In most of the developing solvents examined the R_F values of the substituted phenylthiazolidinones were generally higher than that of the unsubstituted compound, irrespective of the nature of the substituent in the phenyl group, whether electron donating [CH_3 , R, $\text{C}_6\text{H}_4\text{R}$, I or $\text{N}(\text{C}_2\text{H}_5)_2$] or electron withdrawing (NO_2 , OCH_3 , Br or Cl).

The IR stretching frequencies of the characteristic C–N and C=O (ring) groups of thiazolidinones, being highly sensitive to the nature and position of substituents, were correlated with the R_F values in almost all of the developing solvents. In *para*-thiazolidinones both spectral parameters, which are in accord with the electron-repelling ability of the substituents, fall in the orders opposite to those of the R_F values. In isomers the retention orders were different in different systems and similar or opposite to the order of the IR frequencies.

Separation, identification and determination of thiazolidinones in mixtures

Among different solvents tried for the separation of thiazolidinones, benzene showed the highest resolving capacity as it could resolve several mixtures of six compounds; the best resolution of five or less compounds, however, could only be achieved in toluene. For the ternary mixture of nitrothiazolidinones and binary mixtures of naphthyl- and methoxythiazolidinones benzene–ethanol (24:1, v/v) and toluene and ethyl acetate, respectively, are the best resolving solvents. Spectral and R_F correlations were used for the identification of mixture components after separation.

In addition to the solvents listed in Table II, several others were also tried. In acetone, ethanol, acetic acid, dioxane and benzene–ethanol (3:2, 1:1 and 2:3) all the substances had very high R_F values and in carbon tetrachloride and dichloromethane all substances, except the *o*-nitrophenyl compound (R_F 0.07) and all three nitrophenyl compounds (R_F 0.44), respectively remained at the origin.

In order to test the application of the TLC method in the analysis of thiazolidinones, various mixtures were resolved qualitatively on 0.10-cm thick layers (Table II) and a few typical mixtures of isomeric compounds including a ternary mixture of nitrothiazolidinones in benzene–ethanol (24:1, v/v), and binary mixtures of naphthyl- and methoxythiazolidinones in toluene and ethyl acetate, respectively, were analysed quantitatively on 0.15-cm thick layers (Table III). The highest amounts of isomers resolved (Table III) from their mixtures reveal the maximum separation limits of this method. The reproducibility of the results, as can be seen from Table III, is good.

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