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SEPARATION OF MONONITROACETAMIDOTOLUENE AND MONONITROMETHYLPHENOL ISOMERS BY THIN-LAYER AND GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

The separations of mononitroacetamidotoluene and mononitromethylphenol isomers have been studied by thin-layer (TLC) and gas–liquid chromatography (GLC). The nine mononitroacetamidotoluene isomers are separated on silicone FL-100 by GLC, and on a silica gel plate with chloroform–ethyl acetate (9:1) by TLC. The seven mononitromethylphenol isomers are separated on ethylene glycol phthalate by GLC, and on an alumina plate with cyclohexane–acetone (8:2) by TLC. *Ortho* steric effects have been found in these separations.

INTRODUCTION

Although many reports have been published on the separations of the mononitrotoluidine isomers by paper chromatography[1–3] and thin-layer chromatography (TLC)[4], and on the separations of phenol and alkylphenols by gas–liquid chromatography (GLC)[5–9], there have been few studies of the GLC and TLC separations of mononitroacetamidotoluene and mononitromethylphenol isomers which are important raw materials for many kinds of organic compounds. For these reasons, their separations by GLC and TLC were investigated.

EXPERIMENTAL

Gas chromatography

The gas chromatograph was a Shimadzu GC-5A with a flame ionization detector. The chromatographic column (75, 100 or 150 cm × 3 mm I.D., stainless-steel U-tube) was packed with C_{22} or Celite 545 (60–80 mesh) coated with liquid stationary phase (see Table III). The temperature of the column was 180° or 190° and that of the injector was 270°. The flow-rate of the carrier gas (nitrogen) was 20 or 30 ml/min. The flow-rate of hydrogen was 50 ml/min and that of air was 1 l/min for the flame ionization detector. The sample size was 0.5–1.0 μl.

The support comprised Sil-O-Cel C_{22} firebrick (60–80 mesh) (Johns-Manville,

Denver, Colo., U.S.A.) agitated in hot 3 *M* hydrochloric acid for 1 h, then washed repeatedly with water until neutrality and dried prior to use. The C_{22} firebrick was coated with 3 or 20%(w/w) of liquid phase and heated in an electric oven for 16 h in order to remove the solvent at the temperature of the column. The column packed with C_{22} firebrick coated with liquid phase was kept under a stream of nitrogen for 6 h at a temperature *ca.* 20° higher than the column temperature used. The liquid phases silicone KF-54, KF-965 and FL-100 (Shinetsu, Tokyo, Japan), ethylene glycol phthalate (EGP), ethylene glycol isophthalate (EGIP), polyethylene glycol 20M and Apiezon grease L (Gasukuro-Kogyo, Tokyo, Japan) and Bentone-34 were used without purification. The support material (Celite 545, 60–80 mesh) coated with silicone OV-17 (3% w/w) was purchased from Nishio Kogyo (Tokyo, Japan).

Thin-layer chromatography

The glass plates were rinsed in detergent overnight and washed several times with tap water. Silica gel GF_{254} (Type 60) or basic alumina HF_{254} (Type 60/E) (E. Merck, Darmstadt, G.F.R.) (15 g) was suspended in 35 ml of distilled water and the suspension was coated on a glass plate (5 × 15 cm) to a thickness of 250 μm using an applicator made in this laboratory. The thin-layer plates were dried in air at room temperature for 10 h to remove surface water and activated for 1 h at 110° in an electric oven.

A mixture of nine mononitroacetamidotoluene isomers or of seven mononitromethylphenol isomers was examined. A 0.1-μl sample of each solution (10 mg of the mixture dissolved in 10 ml of tetrahydrofuran) was applied to the chromatoplate with a glass capillary. The chromatograms were developed at room temperature in a glass chamber containing 30 ml of solvent mixture (without previous saturation), by one-dimensional ascending chromatography. The development of the solvent front was 13 cm (in Table II) or 10 cm (in Table IV). The detection of the spots was performed with a FI-3S UV lamp (Toshibas, Tokyo, Japan).

The following solvent systems were used for the separation of the mononitroacetamidotoluene isomers: (A) Chloroform–ethyl acetate (9:1); (B) carbon tetrachloride–ethyl acetate–ethanol (85:10:5); (C) cyclohexane–chloroform–ethyl acetate (50:20:20).

In addition to B, the following systems were used for the separation of mononitromethylphenol isomers: (D) carbon tetrachloride–ethyl acetate (9:1); (E) cyclohexane–acetone (8:2).

Samples

4- and 6-nitro-3-acetamidotoluene (4- and 6-N-3-AT) were prepared[10–14] then purified by recrystallization from aqueous ethanol; 6-N-3-AT obtained from an aqueous solvent contains one molecule of water of crystallization[15]. 3- and 5-nitro-2-acetamidotoluene (3- and 5-N-2-AT)[13,16,17] were prepared, separated from each other and purified by recrystallization from aqueous ethanol. 5-Nitro-3-acetamidotoluene (5-N-3-AT)[18], 2-nitro-4-acetamidotoluene (2-N-4-AT)[18] and 4- and 6-nitro-2-acetamidotoluene (4- and 6-N-2-AT)[14,19–21] were obtained from nitroamines by acetylation and 3-nitro-4-acetamidotoluene (3-N-4-AT)[22] was obtained from acetamidotoluene by nitration; they were purified by the procedure mentioned above. All of the samples were purified and their purities were confirmed quantitatively by GLC and qualitative-

ly by TLC and GLC and by IR and NMR spectroscopy. 6-N-3-AT monohydrate was used as a sample of 6-N-3-AT.

5-Nitro-2-methylphenol (5-N-2-MP) was prepared from 4-nitro-2-aminotoluene[23], 2-, 4- and 6-nitro-3-methylphenol (2-, 4- and 6-N-3-MP) from the nitration of *m*-methylphenol[24–26] and 3-nitro-4-methylphenol (3-N-4-MP) from the nitration of *p*-methylphenol[27]. 2-Nitro-4-methylphenol (2-N-4-MP) was prepared from 3-nitro-4-aminotoluene by nucleophilic substitution[28] and 3-nitro-2-methylphenol (3-N-2-MP) was prepared from 6-nitro-2-aminotoluene by the Sandmeyer reaction[29]. All of the samples were purified by recrystallization from aqueous ethanol or ligroin and their identification and purities were confirmed by GLC and TLC, and by IR and NMR spectroscopy.

RESULTS AND DISCUSSION

Separation of mononitroacetamidotoluene isomers

Table I of a previous paper[30] is reproduced here, and shows the results of the separation by GLC of the nine mononitroacetamidotoluene isomers (3- and 4-N-2-AT, 5- and 6-N-2-AT, 4-, 5- and 6-N-3-AT and 2- and 3-N-4-AT). It can be seen that 4-N-3-AT, 3-N-4-AT, 3- and 6-N-2-AT are separated from the other five isomers and from each other, and the other isomers are separated into two groups by GLC on EGP. The elution order of the nine isomers is as follows: 4-N-3-AT; 3-N-4-AT; 3-N-2-AT; 6-N-2-AT; 2-N-4-AT; 4-N-2-AT and 5-N-2-AT; 5-N-3-AT, 6-N-3-AT.

TABLE I

RETENTION TIMES OF MONONITROACETAMIDOTOLUENE ISOMERS ON DIFFERENT LIQUID STATIONARY PHASES

Sample	*Retention time (min)*								
	EGP	*EGIP*	*TPA**	*FL-100*	*KF-96*	*KF-965*	*HV-G**	*OV-17*	*KF-54*
3-N-2-AT	6.80	4.60	3.40	3.40	1.00	2.40	2.20	3.10	6.60
4-N-2-AT	38.80	24.60	21.80	8.40	4.40	5.20	5.40	10.10	20.40
5-N-2-AT	38.80	24.60	16.60	8.40	4.40	5.20	5.40	10.10	20.40
6-N-2-AT	21.00	13.80	14.60	7.40	3.60	4.20	4.60	4.50	13.20
4-N-3-AT	4.60	2.80	2.60	2.00	0.80	2.40	2.20	2.50	6.60
5-N-3-AT	46.20	28.40	21.80	8.40	4.40	5.20	5.40	10.10	20.40
6-N-3-AT	46.20	28.40	14.60	7.40	4.40	5.20	5.40	8.30	18.40
2-N-4-AT	38.80	24.60	16.60	8.40	3.60	4.20	4.60	8.30	18.40
3-N-4-AT	5.20	2.80	3.40	2.20	0.80	2.40	2.20	2.50	6.60
Column length (m)	0.75	0.75	1.00	1.00	1.00	0.75	1.00	1.50	1.00
Flow-rate of carrier gas (ml/min)	20	20	20	20	20	20	20	20	20
Loading (%)	10	10	10	20	20	20	20	3	20

* See ref. 30.

The results of the separation by TLC of the nine mononitroacetamidotoluene isomers are shown in Table II and Fig. 1, where it can be seen that 3-N-4-AT and 4-N-3-AT are separated from the other seven isomers and from each other with solvents A, B and C, and 4-N-2-AT is separated from the other eight isomers with solvents A

TABLE II

R_F VALUES OF MONONITROACETAMIDOTOLUENE ISOMERS ON SILICA GEL PLATES

Sample	R_F		
	Solvent A	*Solvent B*	*Solvent C*
3-N-2-AT	0.42	0.23	0.15
4-N-2-AT	0.29	0.12	0.09
5-N-2-AT	0.37	0.15	0.09
6-N-2-AT	0.25	0.15	0.07
4-N-3-AT	0.85	0.85	0.58
5-N-3-AT	0.37	0.23	0.12
6-N-3-AT	0.35	0.20	0.12
2-N-4-AT	0.35	0.20	0.12
3-N-4-AT	0.80	0.77	0.50

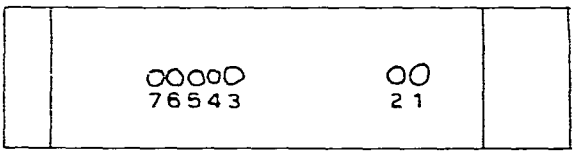

Fig. 1. Separation of mononitroacetamidotoluene isomers on a silica gel plate. Solvent: chloroform–methyl acetate (9:1). Spots: 1 = 4-N-3-AT; 2 = 3-N-4-AT; 3 = 3-N-2-AT; 4 = 5-N-2-AT and 5-N-3-AT; 5 = 6-N-3-AT and 2-N-4-AT; 6 = 4-N-2-AT, 7 = 6-N-2-AT.

and B. 3- and 6-N-2-AT are separated from the other seven isomers and from each other with solvents A and C. Except for 3-N-4-AT and 4-N-3-AT, the separations of the seven isomers were difficult but were improved by using chloroform and ethanol. The characteristic of these separations is that the R_F values of 3-N-4-AT and 4-N-3-AT are very large and those of the other isomers are small. The elution order in the TLC separations (Table II, Fig. 1) is as follows: 4-N-3-AT; 3-N-4-AT; 3-N-2-AT; 5-N-2-AT, 5-N-3-AT; 6-N-3-AT, 2-N-4-AT; 4-N-2-AT; 6-N-2-AT.

Although there are a few differences between the elution orders obtained by GLC and by TLC, there is a general tendency for the isomers in which a nitro group is *ortho* to an acetamido group, *e.g.*, 3-N-4-AT, 4-N-3-AT and 3-N-2-AT, to be eluted faster than the other isomers, and the isomers in which a nitro group is *meta* or *para* to an acetamido group are eluted later than the other isomers. There is *ortho* steric effect in these TLC and GLC separations as proposed by Janák and Komers[31] in the separations of alkylphenols by GLC. Further, the steric effect of nitro *ortho* to acetamido is dominant over that of methyl *ortho* to acetamido. These separations by both GLC and TLC are important for the identification of the nitration products of acetamidotoluenes.

The following conclusions may be made:

(1) EGP is the best liquid phase for the separation of mononitroacetamidotoluene isomers.

(2) The nine isomers are separated into seven spots on a silica gel plate with solvent A.

(3) There is an *ortho* steric effect in these separations by GLC and TLC.

Separation of mononitromethylphenol isomers

The results of the separations of the seven mononitromethylphenol isomers (3- and 5-N-2-MP, 2-, 4- and 6-N-3-MP and 2- and 3-N-4-MP) are shown in Table III and Fig. 2, where it can be seen that 5-N-2-MP, 3-N-2-MP, 4-N-3-MP and 3-N-4-MP are separated from the other three isomers and from each other on KF-54 (25% phenylsilicone), KF-965 (methylsilicone), FL-100 (50% trifluoropropylsilicone), EGIP and EGP, and 2-N-3-MP is separated from the other six isomers on FL-100.

TABLE III

RETENTION TIMES OF MONONITROMETHYLPHENOL ISOMERS ON DIFFERENT LIQUID STATIONARY PHASES

Sample	*Retention time (min)*				
	EGIP	*EGP*	*KF-54*	*FL-100*	*KF-965*
3-N-2-MP	6.70	12.20	14.80	4.60	6.60
5-N-2-MP	10.10	19.40	19.50	6.60	8.70
2-N-3-MP	0.90	1.50	5.20	1.50	3.20
4-N-3-MP	14.90	25.90	21 50	7.30	9.20
6-N-3-MP	0.90	1.50	5.20	1.80	3.20
2-N-4-MP	0.90	1.50	5.20	1.80	3.20
3-N-4-MP	9.50	16.90	16.80	5.60	7.80
Column temperature (°C)	180	190	180	170	190
Column length (cm)	75	75	100	150	150
Flow-rate of carrier gas (ml/min)	30	20	20	20	20
Loading (%)	10	10	20	20	20

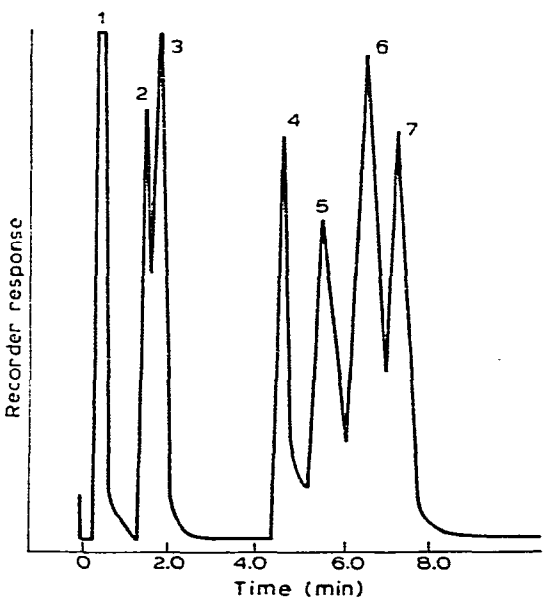

Fig. 2. Separation of mononitromethylphenol isomers on FL-100. Peaks: 1 = tetrahydrofuran (solvent); 2 = 2-N-3-MP; 3 = 6-N-3-MP and 2-N-4-MP; 4 = 3-N-2-MP; 5 = 3-N-4-MP; 6 = 5-N-2-MP; 7 = 4-N-3-MP.

The separations of the four isomers 5-N-2-MP, 3-N-2-MP, 4-N-3-MP and 3-N-4-MP are achieved, but the separation between 5-N-2-MP and 4-N-3-MP is poor on KF-965 and only a little less so on FL-100; the separation between 5-N-2-MP and 3-N-4-MP is poorest on EGIP. The separation of 2-N-3-MP from 2-N-4-MP and 6-N-3-MP is achieved only on silicone FL-100.

From above results it can be seen that the separation of *ortho*-substituted isomers having a similar structure, 2-N-4-MP and 6-N-3-MP, is difficult on these liquid phases. This behaviour of phenols in the GLC separations (*ortho* steric effect) was also proposed by Janák and Komers[31]. Further, the separations of the other four isomers (5-N-2-MP, 3-N-2-MP, 4-N-3-MP and 3-N-4-MP) are achieved on the liquid polysiloxane and polyester phases. It can also be seen that the phenyl group of the liquid phases is effective for the separation between 5-N-2-MP and 4-N-3-MP, and polysiloxanes are effective for the separation of 3-N-4-MP and 5-N-2-MP.

In these separations, the elution order of the isomers is as follows: 2-N-3-MP; 6-N-3-MP, 2-N-4-MP; 3-N-2-MP; 3-N-4-MP; 5-N-2-MP; 4-N-3-MP. The isomers in which a nitro group is *ortho* to an hydroxy group are eluted faster than the other isomers, and the isomers in which a nitro group is *meta* or *para* to an hydroxy group are eluted later than the other isomers.

Unsuccessful attempts were made to separate the seven isomers on other liquid phases (Apiezon grease L, PEG 20M, OV-17 and Bentone-34 mixed liquid phase of 5% KF-54 and 5% Bentone-34). The poor separation on Silicone OV-17 seems to reflect the low loading.

The results of the separations of the seven mononitromethylphenol isomers by TLC are shown in Table IV and Fig. 3, where it can be seen that 2-N-4-MP,

TABLE IV

R_F VALUES OF MONONITROMETHYLPHENOL ISOMERS BY TLC

S = Silica gel plate; A = alumina plate.

Sample	R_F			
	Solvent D	*Solvent B*	*Solvent B*	*Solvent E*
3-N-2-MP	0.47	0.61	0.57	0.41
5-N-2-MP	0.60	0.71	0.67	0.49
2-N-3-MP	0.80	0.82	0.37	0.22
4-N-3-MP	0.35	0.50	0.24	0.13
6-N-3-MP	0.92	0.95	0.74	0.57
2-N-4-MP	0.92	0.95	0.74	0.60
3-N-4-MP	0.47	0.61	0.46	0.34
Kind of plate	S	S	A	A

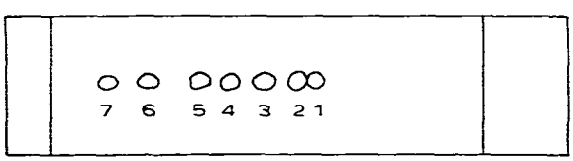

Fig. 3. Separation of mononitromethylphenol isomers on an alumina plate: solvent; cyclohexane–acetone (8:2). Spots: 1 = 2-N-4-MP; 2 = 6-N-3-MP; 3 = 5-N-2-MP; 4 = 3-N-2-MP; 5 = 3-N-4-MP; 6 = 2-N-3-MP; 7 = 4-N-3-MP.

6-N-3-MP, 5-N-2-MP, 3-N-2-MP, 3-N-4-MP, 2-N-3-MP and 4-N-3-MP are separated from each other on an alumina plate with solvent E. In these separations, the elution order is as follows: 2-N-4-MP; 6-N-3-MP; 5-N-2-MP; 3-N-2-MP; 3-N-4-MP; 2-N-3-MP; 4-N-3-MP. With solvent D, 4-N-3-MP, 5-N-2-MP and 2-N-3-MP are separated from the other four isomers and from each other, and 3-N-2-MP and 3-N-4-MP are separated from 3-N-4-MP and 6-N-3-MP on a silica gel plate. In these separations, seven or six separated spots are scattered with approximately equal intervals between them, but two spots are some distance from the other seven isomers in the separations of mononitro-acetamidotoluene isomers. Further, there are a few differences in their elution order mentioned above, 2-N-3-MP being eluted later than 3-N-4-MP on an alumina plate.

In the elution order obtained by TLC, the isomers possessing a nitro group *ortho* to an hydroxy group are eluted faster than the other isomers, and the isomers in which the nitro group is *meta* or *para* to an hydroxy group are eluted later than the other isomers. In the separations of mononitromethylphenol isomers by GLC and TLC, there is also an *ortho* steric effect and the effect of nitro *ortho* to hydroxy is dominant over that of methyl *ortho* to hydroxy. Further, some parts of the elution order on a silica gel plate are changed on an alumina plate. These results depend on a basicity effect.

The following conclusions can be made:

(1) The separation of 2-N-3-MP from 2-N-4-MP and 6-N-3-MP by GLC is achieved on FL-100.

(2) The separations of 5-N-2-MP, 3-N-2-MP, 4-N-3-MP and 3-N-4-MP are achieved on polysiloxanes and polyesters.

(3) The separations of the seven isomers by TLC are achieved on an alumina plate with solvent E.

(4) In the separations of the isomers by GLC and TLC, there are *ortho* steric effects and nitro *ortho* to hydroxy is dominant over methyl *ortho* to hydroxy.

(5) There are basicity effects in the separations by TLC on an alumina plate.

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