

SEPARATION OF 2-BUTYNE-1,4-DIOL,  
ITS MONOESTERS AND DIESTERSA COMPARISON OF GLASS-PAPER CHROMATOGRAPHY AND  
THIN-LAYER CHROMATOGRAPHY

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Glass-paper chromatography and thin-layer chromatography are two chromatographic techniques, recently introduced, which employ adsorption on a micro scale. Both techniques have been applied to the separation of lipids and related compounds. DIECKERT<sup>1,2</sup> and coworkers employed glass-paper chromatography to separate lipids into classes while HOLMAN<sup>3</sup>, MANGOLD<sup>4</sup>, and others<sup>5,6</sup> have applied thin-layer chromatography to the same problem.

To follow stepwise the synthesis and purification of esters of 2-butyne-1,4-diol, glass-paper and thin-layer chromatography methods for separating the individual ester mixtures have been developed for the first five ester homologs. A successful separation of the diol, monoesters, and diesters was achieved by chromatographing the mixtures on treated glass paper or on silica-gel-coated plates. Following development, the spots were detected with alkaline permanganate.

## EXPERIMENTAL

*Glass paper strips*

Sheets of glass paper\*\*, 25 cm × 38 cm, were cut (perpendicular to the grain of the paper) into quarters 9 1/2 cm × 25 cm. Each quarter<sup>7</sup> was immersed in 200 ml of a 2% solution of sodium silicate\*\*\* (10.5 ml sodium silicate, Be 40° and 190 ml distilled water), dried in an oven at 100–110° for 1 h, then immersed in 300 ml of 4 N hydrochloric acid for 5 min. Following the acid bath, the paper was washed in 200 ml of distilled water for 5 min six successive times<sup>2</sup>. The paper was dried at 100–110° for 2 h then marked vertical to the base with a grease-free lead pencil at 3, 21, and 24 cm. Finally, each quarter of paper was cut into six strips, 1.5 cm × 25 cm. The individual strips were washed with the developing solvent to the 24 cm mark, ascending technique, dried over a hot plate, and spotted at the 3 cm mark on the smooth side of the paper. To determine the best conditions for separation, two mixtures of esters and diol: 2-butyne-1,4-diol, monoacetate, and diacetate, and 2-butyne-1,4-diol, the monovalerate, and divalerate were chromatographed.

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\*\* Reeves Angel, No. 934-AH.

\*\*\* Philadelphia Quartz, lot No. 60948P.

### Development

Glass columns 40 cm × 4.1 cm served as the developing chamber. The columns were equilibrated with developing solvent at  $27^{\circ} \pm 1^{\circ}$  for 4 h prior to the insertion of the spotted paper. To determine the solvent front, the chromatograms were viewed with indirect light. The chromatograms were developed to the 21 cm line, dried over a hot plate, then sprayed with the detecting reagent. All  $R_F$  values reported are the average of three determinations.

### Conditions studied

The effect of each of the following conditions on the  $R_F$  of the esters was investigated:

1. Concentration of silicate coating: 0.25, 0.5, 1.0, and 2.0 %.
2. Solvent composition: isooctane-isopropyl ether: 100:0, 95:5, 90:10.
3. Heat-cleaned glass paper\* as the silicic acid matrix *versus* glass paper not heat cleaned.
4. Variation of the sodium silicate from batch to batch.
5. Variation of the glass paper from sheet to sheet and lot to lot.

### Thin-layer silica gel plates

Ten glass plates, 6 cm × 20 cm, were coated with a mixture of 20 g silica gel, Brockmann G\*\* in 40 ml of distilled water, air-dried for 30 min, then activated at 100–110° for 1 h. The plates were stored in a desiccator until required for use. The starting points (3 cm from the lower edge of the plate and at a distance of 1 cm from each other) and a fine line at a distance 15 cm from these points were marked on the plate with a needle. Each plate was spotted with the five ester mixtures. Solvent was placed in the bottom of a glass tank (12 in. × 12 in. × 3 in.) to a height of 1 cm and adsorbent paper moistened with the solvent was placed on the sides of the tank. The tank was equilibrated 1 h before use. Individual plates were placed in the tank and development carried out until the solvent front reached the marked line. The temperature was maintained at  $27^{\circ} \pm 1^{\circ}$  throughout. Six solvent systems were investigated.

### Solvents

Isopropyl ether, washed three times with 4 % ferrous ammonium sulfate, three times with water, dried over calcium chloride and distilled, b.p. 67–69°. Isooctane washed three times with concentrated sulfuric acid, three times with water, dried over calcium chloride and distilled, b.p. 98–99°. Each of the following predried solvents was distilled: benzene, b.p. 79–81.5°; chloroform, b.p. 61–62°; ethyl acetate, b.p. 76–77°. Diethyl ether, Mallinkrodt, anhydrous, reagent grade.

### Ester solutions

Five solutions, each a mixture of equal parts of 2-butyne-1,4-diol, monoester, and diester were prepared. The composition of the solutions was:

\* Sheets of glass paper were heat cleaned in a muffle furnace for 1 h at 600° prior to impregnating.

\*\* Batch No. 62551.

2-butyne-1,4-diol, monoformate and diformate  
2-butyne-1,4-diol, monoacetate and diacetate  
2-butyne-1,4-diol, monopropionate and dipropionate  
2-butyne-1,4-diol, monobutyrate and dibutyrate  
2-butyne-1,4-diol, monovalerate and divalerate.

The synthesis of the esters will be reported elsewhere.

*Concentration.* 1 % w/v with respect to each component in benzene. 2  $\mu$ l of each solution was spotted on the individual paper strip or plate.

#### *Detection of spots on glass paper and silica-gel plates*

After development the paper or plate was sprayed with a solution containing 0.5 % potassium permanganate in 2 % sodium hydroxide. On the glass paper the compounds first appeared as green spots on a lavender background. After a few minutes the spots turned yellow. It is essential to mark the spots promptly because the background of the strip changes from lavender to yellow within a short time after spraying. In the case of the silica-gel plates yellow spots appeared on a lavender background. These spots faded less rapidly.

### RESULTS AND DISCUSSION

#### *Glass-paper chromatography*

Results of the preliminary investigation to determine the conditions necessary for separating the esters of 2-butyne-1,4-diol are summarized in Table I. In this investigation, the greatest source of variability affecting the  $R_F$  values was the glass paper. There was variation from sheet to sheet and from lot to lot. Also, there was some variation in commercial silicate solutions from one batch to another. Reproducibility of the  $R_F$  value for a given compound separated from a mixture on treated glass paper was good only if all conditions were carefully maintained and the strips from the same sheet of glass paper were used. Equilibrating the paper (2 to 24 h) after spotting and prior to development adversely affected the size of the spots. In such cases the  $R_F$  values varied considerably and the spots were diffuse and of poor quality. Some development by the vapors of the solvent probably accounts for the erratic behavior. Equilibrating the columns for 4 h before inserting the paper in the developing chamber greatly improved the quality of the spots.

*Results with glass paper not heat-cleaned.* Paper treated with 0.25 % sodium silicate solution was very soft and the  $R_F$  values for the esters separated on this paper with isooctane-isopropyl ether (95:5) were high. There was little difference in the  $R_F$  values of the components separated on 0.5\*, 1.0, or 2.0 % silicate-treated paper with isooctane-isopropyl ether (95:5) as the developing solvent. Only with 2 % silicate coated paper was the migration of the divalerate slow enough to keep the compound off the solvent front.

With isooctane as the developing solvent and 2 % silicate-treated paper, the  $R_F$  values determined were low and the spots long and elliptical. The monoester spots averaged 3.5 cm in length and the diester spots 4.3 cm. With isooctane-isopropyl ether (90:10) the divalerate was always located on the solvent front.

\* With all three concentrations the treated paper was hard.

TABLE I

RESULTS OF AN INVESTIGATION OF CONDITIONS FOR SEPARATING ESTERS OF 2-BUTYNE-1,4-DIOL BY GLASS PAPER CHROMATOGRAPHY

Condition varied	$R_F \times 100$						Time <sup>a</sup> (min)
	Acetate esters			Valerate esters			
	Diol	Mono	Di	Diol	Mono	Di	
<i>Conc. of coating soln.<sup>b</sup></i>							
0.25% Sodium silicate	2 ± 0	28 ± 2	84 ± 1	0 ± 0	60 ± 3	96 ± 2	35 ± 2
0.5% Sodium silicate	0 ± 0	14 ± 2	63 ± 3	0 ± 0	45 ± 2	95 ± 1	31 ± 2
1.0% Sodium silicate	0 ± 0	13 ± 1	64 ± 4	0 ± 0	44 ± 1	98 ± 2	33 ± 1
2.0% Sodium silicate	0 ± 0	12 ± 0	61 ± 1	0 ± 0	42 ± 1	94 ± 2	30 ± 1
<i>Developing solvent<sup>c</sup></i>							
Isooctane	0 ± 0	9 ± 1	34 ± 6	0 ± 0	14 ± 2	61 ± 3	33 ± 4
Isooctane, isopropyl ether 95:5	0 ± 0	17 ± 1	60 ± 1	0 ± 0	32 ± 2	89 ± 1	33 ± 4
Isooctane, isopropyl ether 90:10	3 ± 0	39 ± 0	79 ± 2	2 ± 1	55 ± 1	96 ± 1	29 ± 3
<i>Other factors<sup>d</sup></i>							
Paper heat-cleaned	0 ± 0	30 ± 2	77 ± 1	0 ± 0	46 ± 0	92 ± 0	30 ± 1
Paper not heat-cleaned	2 ± 0	33 ± 1	80 ± 1	1 ± 0	49 ± 1	94 ± 1	33 ± 3
Another batch of silicate <sup>e</sup>	3 ± 0	38 ± 2	84 ± 3	2 ± 0	60 ± 3	97 ± 0	37 ± 3
<i>Reproducibility of <math>R_F</math> values</i>							
Stock sheet 1	0 ± 0	12 ± 0	61 ± 1	0 ± 0	42 ± 1	94 ± 2	30 ± 1
Stock sheet 2	0 ± 0	17 ± 1	60 ± 1	0 ± 0	32 ± 2	89 ± 1	33 ± 4
Stock sheet 3	2 ± 0	33 ± 1	80 ± 1	1 ± 1	49 ± 1	94 ± 1	33 ± 3
Stock sheet from another lot of paper	0 ± 0	20 ± 3	69 ± 2	0 ± 0	36 ± 0	91 ± 0	30 ± 2

The following conditions apply to all the above determinations except as noted: glass paper (same lot), not heat-cleaned, coated with 2% sodium silicate. Developing solvent: Isooctane-isopropyl ether (95:5). Temperature  $27^\circ \pm 1^\circ$ . Solvent height 18 cm. All  $R_F$ 's the average of 3 determinations.

<sup>a</sup> Development time

<sup>b</sup> All strips from one sheet of stock paper (not heat-cleaned).

<sup>c</sup> All strips from one sheet of stock paper (not heat-cleaned).

<sup>d</sup> All strips from one sheet of stock paper.

<sup>e</sup> Philadelphia Quartz, sodium silicate no. 60347P.

*Results with heat-cleaned glass paper.* When the glass paper was heat-cleaned prior to coating with 2% sodium silicate solution, the  $R_F$  values were slightly lower and the quality of the spots improved. Presumably, some annealing of the paper occurs on heat-cleaning. This makes the paper more dense and compact in structure. The shape of the developed spot varied with the polarity of the compound; the diol was always small and compact, the monoesters formed elliptical spots averaging 3.3 cm long and 1 cm wide, while the diesters formed oval spots averaging 2.0 cm long and 1 cm wide.

Optimum conditions for separating these compounds are heat-cleaned glass paper treated with 2% sodium silicate. The solvent system, isooctane-isopropyl ether (95:5), provided good separation of the compounds without the divalerate ascending to the solvent front.

TABLE II  
GLASS-PAPER CHROMATOGRAPHY  
INCREASE OF  $R_F$  WITH INCREASED CONCENTRATION APPLIED

Ester	Concentration					
	1/8 %	1/4 %	1/2 %	1 %	5 %	10 %
Monoacetate	19 ± 0	20 ± 1	21 ± 3	31 ± 2	36 ± 2	36 ± 1
Diacetate	64 ± 1	65 ± 2	68 ± 1	71 ± 1	76 ± 2	78 ± 1

Heat-cleaned glass paper, coated with 2 % sodium silicate. Developing solvent: Isooctane-isopropyl ether (95:5). Solvent height 18 cm. Temperature  $27^\circ \pm 1^\circ$ . All  $R_F$ 's the average of 3 determinations. 2  $\mu$ l of solution applied in each case.

With the acetate-diol mixture, the minimum amount which can be detected on heat-cleaned, 2 % silicate-treated glass paper is 2.5  $\mu$ g of each component while the range in concentration of components which can be separated and detected is 2.5  $\mu$ g to 100  $\mu$ g. With concentrations above 100  $\mu$ g of each component the spots are so large that differentiation between the diol and monoester is lost. There is a distinct and stepwise increase in the  $R_F$  of both monoester and diester with increasing concentration of the mixture used to spot the paper (see Table II).

$R_F$  values for all five homologs of the monoesters and diesters of 2-butyne-1,4-diol separated on strips of glass paper, 9 cm  $\times$  25 cm, which had been heat-cleaned at  $600^\circ$  for 1 h and treated with 2 % sodium silicate solution as previously indicated are summarized in Table III. The strips were developed with isooctane-isopropyl ether (95:5), ascending technique, in the same tank used for developing the thin-layer plates. The tank was equilibrated one hour before developing the spotted strips.

TABLE III  
 $R_F$  VALUES  $\times$  100 OF ESTERS OF 2-BUTYNE-1,4-DIOL AT OPTIMUM SOLVENT COMPOSITION

Ester	Glass-paper chromatography <sup>a</sup>				Thin-layer chromatography <sup>b</sup>			
	Diol	Mono	Di	$\Delta R_F$	Diol	Mono	Di	$\Delta R_F$
Formate	0 ± 0	17 ± 7	66 ± 1	49	5 ± 0	23 ± 1	47 ± 4	24
Acetate	0 ± 0	23 ± 3	68 ± 2	45	5 ± 0	22 ± 1	36 ± 2	14
Propionate	0 ± 0	30 ± 2	77 ± 2	47	5 ± 0	29 ± 1	57 ± 2	28
Butyrate	0 ± 0	39 ± 1	84 ± 1	45	5 ± 0	34 ± 3	62 ± 2	28
Valerate	0 ± 0	42 ± 3	89 ± 1	47	5 ± 0	37 ± 3	65 ± 2	28

Development time in min		31 ± 2	37 ± 3
Temperature		27 ± 1°	27 ± 1°
Solvent height in cm		18	15
Developing solvent	Isooctane-isopropyl ether (95:5) Paper-lined tank All $R_F$ 's the average of three determinations		Isopropyl ether Paper-lined tank All $R_F$ 's the average of three determinations

<sup>a</sup> 2 % Silicate-treated paper, heat-cleaned.

<sup>b</sup> Brockmann silica gel B batch No. 63131.

TABLE IV

 $R_F \times 100$  OF ESTERS OF 2-BUTYNE-1,4-DIOL SEPARATED BY THIN-LAYER CHROMATOGRAPHY

Compound	Developing solvent <sup>a</sup>				
	Benzene	Chloroform	Isopropyl ether- isooctane (1:1)	Isopropyl ether	Ethyl acetate- isooctane (1:1)
2-Butyne-1,4-diol	0 <sup>b</sup> ± 0	0 ± 0	0 ± 0	5 ± 0	8 ± 1
Monoformate	0 <sup>b</sup> ± 0	4 ± 0	7 ± 1	24 ± 1	28 ± 1
Diformate	12 ± 3	27 ± 0	24 ± 2	48 ± 1	51 ± 4
Monoacetate	0 <sup>b</sup> ± 0	4 ± 0	7 ± 1	20 ± 1	27 ± 2
Diacetate	8 ± 1	22 ± 2	23 ± 2	41 ± 1	48 ± 3
Monopropionate	0 <sup>b</sup> ± 0	4 ± 1	10 ± 1	28 ± 1	34 ± 2
Dipropionate	13 ± 1	27 ± 3	38 ± 2	59 ± 1	60 ± 1
Monobutyrate	3 ± 0	4 ± 1	13 ± 1	32 ± 1	38 ± 1
Dibutyrate	18 ± 2	36 ± 1	45 ± 3	64 ± 1	65 ± 0
Monovalerate	3 ± 0	5 ± 1	14 ± 1	35 ± 1	40 ± 1
Divalerate	23 ± 1	44 ± 4	51 ± 2	69 ± 0	69 ± 1
Developing time in min	45 ± 3	53 ± 2	48 ± 3	37 ± 1	48 ± 1

Developing height 15 cm. Temperature 27° ± 1°. All  $R_F$ 's the average of three determinations. Paper-lined tank.

<sup>a</sup> Brockmann silica gel batch No. 62551.

<sup>b</sup> No separation of diol and monoester.

#### Thin-layer chromatography on silica-gel-coated plates

Model experiments were performed with five different three component mixtures of 2-butyne-1,4-diol, the monoesters, and diesters. Six different solvent systems were employed. The results are summarized in Table IV. The separation of the five mixtures increased with increasing polarity of the solvent. Ethyl acetate-isooctane (1:1) or isopropyl ether produced excellent separations. Diethyl ether was unsatisfactory as the developing solvent. With isopropyl ether as the solvent, the developing time was

TABLE V

SEPARATION OF ESTERS OF 2-BUTYNE-1,4-DIOL BY THIN-LAYER CHROMATOGRAPHY  
REPRODUCIBILITY OF  $R_F$  VALUES

Ester	Silica gel G <sup>a</sup>			Silica gel G <sup>b</sup>		
	Diol	Mono	Di	Diol	Mono	Di
Formate	5 ± 0	24 ± 1	48 ± 1	5 ± 0	23 ± 1	47 ± 4
Acetate	5 ± 0	20 ± 1	41 ± 1	5 ± 0	22 ± 1	36 ± 2
Propionate	5 ± 0	28 ± 1	59 ± 1	5 ± 0	29 ± 1	57 ± 2
Butyrate	5 ± 0	32 ± 1	64 ± 1	5 ± 0	34 ± 3	62 ± 2
Valerate	5 ± 0	35 ± 1	69 ± 0	5 ± 0	37 ± 3	65 ± 2
Developing time in min	37 ± 1			37 ± 3		

Developing solvent isopropyl ether. Solvent height 15 cm. Temperature 27° ± 1°. Paper-lined tank. All  $R_F$ 's the average of 3 determinations.

<sup>a</sup> Brockmann batch No. 62551.

<sup>b</sup> Brockmann batch No. 63131.

considerably shorter. In all cases, except diethyl ether, the diol spots averaged 0.5 cm in diameter and the monoester and diester spots were 0.8 cm each in diameter. Reproducibility of  $R_F$  values was good and did not vary greatly from one batch of silica gel to another if care was taken to protect the activated plates from the atmosphere (see Table V). With the acetate mixture the range in concentration which can be separated and detected is  $5 \mu\text{g}$  to  $750 \mu\text{g}$  of each component. The  $R_F$  values did not vary significantly over the entire range in concentration. By lining the developing tank on both sides with adsorbent paper the developing time was cut from  $75 \pm 5$  to  $37 \pm 1$  min. A comparison of results obtained with treated glass paper and silica-gel-coated plates are summarized in Table III. The change in  $R_F$  with increasing chain length of the hydrocarbon group is shown in Fig. 1.

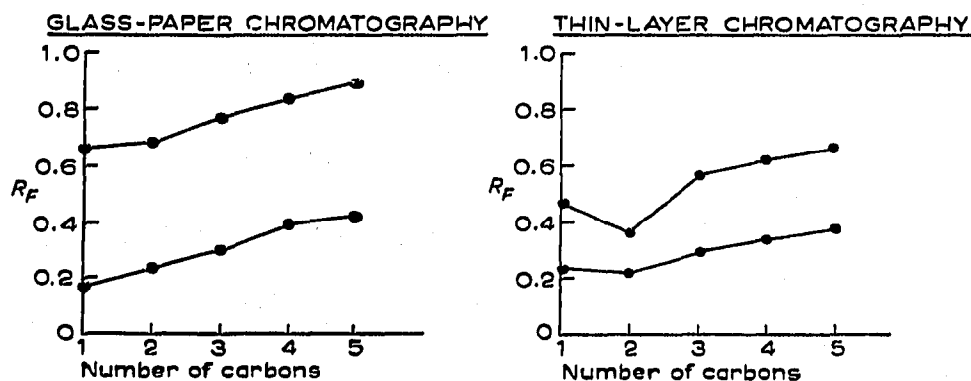


Fig. 1. Variation of  $R_F$  value with hydrocarbon chain length for the homologous series of monoesters and diesters of 2-butyne-1,4-diol. Data from Table III.

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#### SUMMARY

Excellent separations of the five glycol ester mixtures was achieved with treated glass paper and with silica-gel-coated plates. In the separation of 2-butyne-1,4-diol from its monoesters and diesters, thin-layer chromatography offers several advantages over glass-paper chromatography, specifically:

1. The spots are uniformly smaller and more compact.
2. No tailing was ever observed with silica-gel-coated plates, however, on treated glass paper all the monoesters tailed regardless of the conditions employed.
3. The reproducibility of  $R_F$  values from plate to plate and from one lot of silica gel to another is good, significantly better than the reproducibility achieved with different sheets of glass paper.
4. The time required to coat the plates is very short compared to the time required to treat glass paper.
5. The useful range for separating these esters by thin-layer chromatography is seven fold the useful range that obtained with treated glass paper.
6. The  $R_F$  values of the acetate esters separated on silica-gel-coated plates were reasonably constant and did not vary significantly with concentration. The  $R_F$  values

for the acetate esters separated on 2 % sodium silicate-treated glass paper gradually increased with increasing concentration of the solution applied to the paper. This observation suggests that the adsorption isotherm for ester separated on silica-gel plates is linear, whereas the adsorption isotherm for the esters separated on treated glass paper is convex.

7. There is significant interaction of both the monoacetate and diacetate with the silica-gel substrate on the silica-gel plates.

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