

SEPARATION OF FATTY ACETYLENIC, ETHYLENIC AND SATURATED COMPOUNDS BY THIN-LAYER CHROMATOGRAPHY

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(Received February 10th, 1964)

Acetylenic compounds are being increasingly used as intermediates in fatty acid synthesis. Methods for their identification in the presence of corresponding ethylenic and saturated materials are scarce. BALLANCE AND CROMBIE¹ used reversed-phase paper chromatography, which takes about 20 hours, for their separation. In this paper, resolutions by thin-layer chromatography (TLC) of a series of purified fatty acids, esters and alcohols, comprising acetylenic, ethylenic and saturated compounds have been studied both by direct and reversed-phase TLC. Useful systems for specific requirements can be deduced from the results.

MATERIALS AND METHODS

All compounds were of high purity. The ethylenic acids, erucic, oleic, petroselinic and undecenoic, were prepared from mustard, olive, parsley seed and castor oils respectively. Bromination and dehydrobromination² gave the corresponding acetylenic acids, and hydrogenation in ethanol with a-Pd/C catalyst gave the corresponding saturated acids; these derived products were thereafter purified by repeated crystallisation. The acids were converted using methanol-sulphuric acid to methyl esters, and reduced with lithium aluminium hydride in tetrahydrofuran³ to the alcohols, unconverted acids being washed away with alkali.

The direct TLC procedure in use in this laboratory, employing Desaga equipment, plates of silica gel G, and ether-light petroleum for development, has been described previously⁴. For reversed-phase TLC, the dried, coated plate was uniformly impregnated with silicone oil (Dow Corning-silicone fluid, 200) by allowing a 5% solution in ether to ascend the plate in a developing chamber (S. VENKOB RAO, unpublished). This procedure has been found superior to the immersion technique⁵, which requires a large volume of solution and often results in loosening of silica gel from the plate. With acids and esters, only one solvent system was used for reversed-phase TLC, *viz.*, 70% acetonitrile, 10% acetic acid and 20% water (v/v). With alcohols, in addition to this solvent system, three others were also used, *viz.*, 70, 80 and 90% aqueous acetic acids. These acetic acid systems were also tried with acids and esters, but since only the acetylenic and ethylenic products were resolved, results are reported for the acetonitrile system only.

TABLE I
SEPARATION OF ACIDS, METHYL ESTERS AND ALCOHOLS BY DIRECT AND REVERSED-PHASE THIN-LAYER CHROMATOGRAPHY
(All values as $R_F \times 100$)

Chain length	Compound	Place of unsaturation	Common name of acid	Acids			Methyl esters			Alcohols		
				Direct, 5% ether-pet. ether	Reversed-phase, acetonitrile-acetic acid-water (70:10:20)	Direct, 2% ether-pet. ether	Reversed-phase, acetonitrile-acetic acid-water (70:10:20)	Direct, 20% ether-pet. ether	Acetonitrile-acetic acid-water (70:10:20)	Acetic acid-water (70:30)	Acetic acid-water (80:20)	Acetic acid-water (90:10)
22	Behenolic	13,14	43	40	60	14	64	21	7	15	28	
18		9,10	34	64	58	39	61	38	18	26	43	
18		6,7	34	62	58	40	61	38	18	26	43	
11		10,11	19	87	39	81	39	71	44	55	61	
22	Erucic	13,14	52	20	74	7	58	15	3	9	18	
18		9,10	41	50	73	29	54	28	12	18	34	
18	Petroselinic	6,7	41	52	73	30	54	28	12	18	34	
11		10,11	26	75	57	70	34	55	34	44	51	
18	Linolenic	9,10; 12,13	—	62	—	40	54	38	18	26	43	
18		9,10; 12,13; 15,16	—	71	—	50	54	48	23	35	53	
22	Behenic	nil	52	3	74	0	58	0	0	0	4	
18		nil	45	40	71	14	54	21	7	15	28	
11	Undecanoic	nil	28	66	57	60	34	38	26	32	38	
20		nil	52	20	71	7	—	—	—	—	—	
16	Palmitic	nil	44	50	73	29	—	28	12	18	34	
14		nil	44	64	73	39	—	38	18	26	43	
9	Pelargonic	nil	28	75	59	70	—	—	—	—	—	
12		nil	—	71	—	50	—	48	23	35	53	
22	Behenic	nil	52	3	74	0	58	0	0	0	4	
18		nil	45	40	71	14	54	21	7	15	28	
11	Undecanoic	nil	28	66	57	60	34	38	26	32	38	
20		nil	52	20	71	7	—	—	—	—	—	
16	Palmitic	nil	44	50	73	29	—	28	12	18	34	
14		nil	44	64	73	39	—	38	18	26	43	
9	Pelargonic	nil	28	75	59	70	—	—	—	—	—	
12		nil	—	71	—	50	—	48	23	35	53	

TABLE II
DEGREES OF SEPARATION OBTAINED BY THIN-LAYER CHROMATOGRAPHY AND METHODS OF CHOICE

Separation of compounds	Item No.	Acids			Esters			Alcohols			Recommended method Compound and system ^b
		Reversed-phase			Direct			Reversed-phase ^a			
		Direct	Reversed-phase	Direct	Reversed-phase	Direct	Reversed-phase	Direct	Reversed-phase	Direct	
<i>Same chain length</i>											
Acetylenic from ethylenic	1	Poor	Good	Excellent	Fair	Poor	Fair	Fair	Esters, D, 2% ether		
Ethylenic from saturated	2	Impossible	Good	Impossible	Good	Impossible	Good	Good	Esters, RP, 70% acetonitrile		
Acetylenic from saturated	3	Poor	Excellent	Fair	Excellent	Poor	Good	Good	Esters, RP, 70% acetonitrile		
<i>Similar material of various chain lengths</i>											
Acetylenics	22 from 18	Fair	Good	Poor	Good	Poor	Good	Fair	Esters, RP, 70% acetonitrile		
	18 from 11	Good	Good	Poor	Excellent	Very Good	Excellent	Excellent	Esters, RP, 70% acetonitrile		
Ethylenics	22 from 18	Poor	Excellent	Poor	Good	Poor	Fair	Fair	Acids, RP, 70% acetonitrile		
	18 from 11	Fair	Excellent	Fair	Excellent	Very Good	Excellent	Excellent	Esters, RP, 70% acetonitrile		
Saturated	22 from 18	Fair	Excellent	Poor	Fair	Poor	Good	Good	Acids, RP, 70% acetonitrile		
	18 from 11	Fair	Excellent	Fair	Excellent	Very Good	Fair	Fair	Esters, RP, 70% acetonitrile		
<i>Certain difficult pairs</i>											
Acetylenic from saturated of 4C less	10	Impossible	Impossible	Good	Impossible	Poor	Impossible	Impossible	Esters, D, 2% ether		
Ethylenic from saturated of 2C less	11	Impossible	Impossible	Impossible	Impossible	—	Impossible	Impossible	None		
Saturated from next homologue	12	Impossible	Fair	Impossible	Fair	Impossible	Fair	Fair	Esters or Acids, RP, 70% acetonitrile		
One acetylenic from two ethylenics	13	—	Impossible	—	Impossible	Fair	Impossible	Impossible	Alcohols D, 20% ether		
Two ethylenics from three ethylenics	14	—	Fair	—	Fair	Impossible	Fair	Fair	Acids, esters or alcohols, RP, 70% acetonitrile		
<i>Common C₁₈ natural mixture</i>											
Stearic, oleic, linoleic, linolenic	15	Impossible	Fair	Impossible	Good	Impossible	Good	Fair	Esters, RP, 70% acetonitrile		
Unresolved pairs, item numbers		2,10,11,12,15	10,11,13	2,11,12,15	10,11,13	2,12,14,15	10,11,13	10,11,13			

^a Best of several solvent systems tried.

^b D = Direct, RP = reversed-phase; composition of systems only identified briefly, for details see Table I.

^c Positional isomers, acetylenic or ethylenic, are also not resolved by any of the systems tried.

RESULTS AND DISCUSSION

In Table I the results of separation of the various acetylenic, ethylenic and saturated acids, methyl esters and alcohols examined are shown, in terms of $R_F \times 100$ values. Table II evaluates these separations under various classified categories, using an arbitrary descriptive code based on $R_F \times 100$ values as follows: 0-2 impossible; 2-5 poor; 5-10 fair; 10-20 good; and over 20 excellent. Methods of choice for these compounds are shown, while those for specific separations can be deduced.

In direct systems, separations as acids and as alcohols are comparable, but ester separations stand slightly apart. Thus in three instances, direct TLC is the preferred separation method: (a) for resolution of acetylenic from ethylenic esters of the same chain length, *e.g.* stearyl from oleate; here a good reversed-phase system using acids is also available; (b) for separation of an acetylenic from a saturated acid shorter in chain length by four carbon atoms; and (c) for separation of an acetylenic from a diethylenic alcohol, *e.g.* stearyl from linoleyl. In the latter two instances, all other direct and reversed-phase systems are inadequate.

Generally reversed-phase systems are preferable to direct systems for resolution of these compounds. Thus in Table II a larger number of pairs are unresolvable by direct TLC in each category. By reversed-phase TLC, acids, esters and alcohols all show similar separation patterns. In several instances, *e.g.* for separation of oleic from stearic or undecenoic, or of behenic from stearic and from undecylenic, or of various homologous saturated products, esters are more advantageous than acids or alcohols for direct TLC separation. Sometimes the difference between the use of esters and acids or alcohols is only marginal, and either can be employed.

In separating fatty alcohols by reversed-phase TLC, a simple 90 % acetic acid system gives separations similar to those with a 70 % acetonitrile, 10 % acetic acid and 20 % water system.

Separation of a mixture of common C_{18} fatty acids, stearic, oleic, linoleic and linolenic, can best be effected by reversed-phase TLC of the esters using 70 % acetonitrile, 10 % acetic acid and 20 % water. Palmitic is critical with oleic and myristic with linoleic. Mixtures which include all these acids are best determined as esters by resolution (a) as such, (b) after complete hydrogenation, and (c) after conversion of unsaturated acids into bromo- or hydroxy derivatives, as recently suggested⁶.

The following example illustrates the usefulness of these TLC procedures. Quantitative hydrogenation of 9,10-stearic and 13,14-behenic acids with LINDLAR catalyst⁷ till two atoms of hydrogen had been taken up gave the corresponding ethylenic acids. Reversed-phase TLC and column chromatography of the reduction products showed the presence of only ethylenic acids. In addition, the specificity of LINDLAR catalyst was shown by the *cis* configuration of the ethylenic acid, inferred from the absence of a peak at 10.36μ in the infrared spectra of the corresponding esters.

SUMMARY

Direct or reversed-phase thin-layer chromatography was used to separate acetylenic, ethylenic and saturated acids, methyl esters and alcohols of 22, 18 and 11 carbon chain lengths. Products corresponding to pelargonic, myristic, palmitic, arachidic,

linoleic and linolenic acids were also included. Systems are described which will resolve: (a) compounds of the same chain length but of different types of unsaturation; (b) compounds of different chain length but carrying the same type of unsaturation; and (c) certain difficult pairs, such as various saturated homologues. Palmitic and oleic acids were critical under all conditions, as were positional isomers. Thin-layer chromatography, column chromatography and infrared spectroscopy were used to confirm the specificity of LINDLAR catalyst for acetylenic to *cis*-ethylenic reduction.

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