CHROM. 3793

Separation of aliphatic dibasic acids by thin-layer chromatography

During a study on the oxidation of fatty acids, it was necessary to identify the dibasic acids produced by oxidative cleavage. Thin-layer chromatography (TLC) proved to be a useful tool for the solution of this problem. The separation of aliphatic dibasic acids by the TLC method has been studied by several investigators[1–5]. In their methods, silica gel[1–4], silica gel–kieselguhr and cellulose[4], or polyethylene glycol[5] was used as the stationary phase.

In the present communication, a TLC method is reported for the separation of aliphatic dibasic acids (C_2–C_{10}) on a silica gel–kieselguhr layer impregnated with vapors of aqueous acetic acid.

Experimental

Reagents. The coating material was a mixture of Silica Gel G (Merck, Item No. 7731) and Kieselguhr G (Merck, Item No. 8129) (1:3). The pre-treating solvent was 30, 40 or 50% aqueous acetic acid. The developing solvent was an upper layer of a mixture of benzene–80% aqueous acetic acid (100:17.5, v/v) equilibrated at the developing temperature. The chromogenic reagent was 0.2% bromocresol green alcoholic solution adjusted to pH 6.

Preparation of the thin layer. The coating material (10 g) was shaken vigorously with 20 ml of water for 20–30 sec, and the slurry was spread over glass plates (20 × 20 cm) to a thickness of 250 μ with the Desaga equipment. The coated plates were air dried at room temperature, and stored in a box over silica gel until use.

Procedure. The pre-treating solvent (50 ml) was poured into a pre-treating tank (an ordinary chromatographic tank) containing a plate support, and the tank atmosphere was allowed to equilibrate with the solvent vapor for more than 30 min. A glass boat (21 cm × 3.5 cm, height 2 cm), which contained the developing solvent, was set into a developing tank, and the tank atmosphere was allowed to equilibrate with the solvent vapor for more than 30 min. Sample solutions (0.1–0.2% dibasic acid acetone solution, 5–10 μl) were spotted about 1.5 cm from the edge of the plate. The plate was leaned against the side of the pre-treating tank, and a thin layer was impregnated with the vapor of aqueous acetic acid for 30 min. The plate was then transferred immediately into the developing tank and developed with 130 ml of the developing solvent (14 cm/40 min). The plate was dried at 70–80° for 30 min, cooled, and sprayed with the chromogenic reagent.

Results

Table I presents the $R_F \times 100$ values obtained under various conditions. Each value is the average of more than two developments, and the deviation from each average is ± 2–4%.

The R_F values vary slightly according to the temperature and the acid concentration in the pre-treating solvents. However, each chromatogram obtained under the conditions prescribed in Table I indicated satisfactory results for the identification. A typical chromatogram is shown in Fig. 1.

TABLE I

$R_F \times 100$ VALUES OF ALIPHATIC DIBASIC ACIDS FROM OXALIC ACID TO SEBACIC ACID

Temperature (± 1.5°)	*AcOH in pre-treating solvent (%)*	$R_F \times 100$								
		C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
18°	30	0	2.4	13	23	40	58	69	79	86
	40	0	3.0	14	25	40	56	71	80	87
	50	0	3.2	16	29	45	60	71	80	87
25°	30	0	3.0	16	29	45	61	73	83	89
	40	0	3.1	17	31	48	64	75	83	88
	50	0	3.6	19	33	49	64	76	85	90
31°	30	0	3.3	17	31	47	65	80	89	93
	40	0	4.0	20	34	51	67	81	88	93
	50	0	5.0	24	39	54	69	80	87	91

Pre-treatment was carried out to prepare a stationary phase on the thin layers. It was observed that the separation was better and more regular on a treated plate than on an untreated plate. It may be assumed that partition between the absorbed aqueous acetic acid in the layer and the developing solvent makes an important contribution to the degree of resolution.

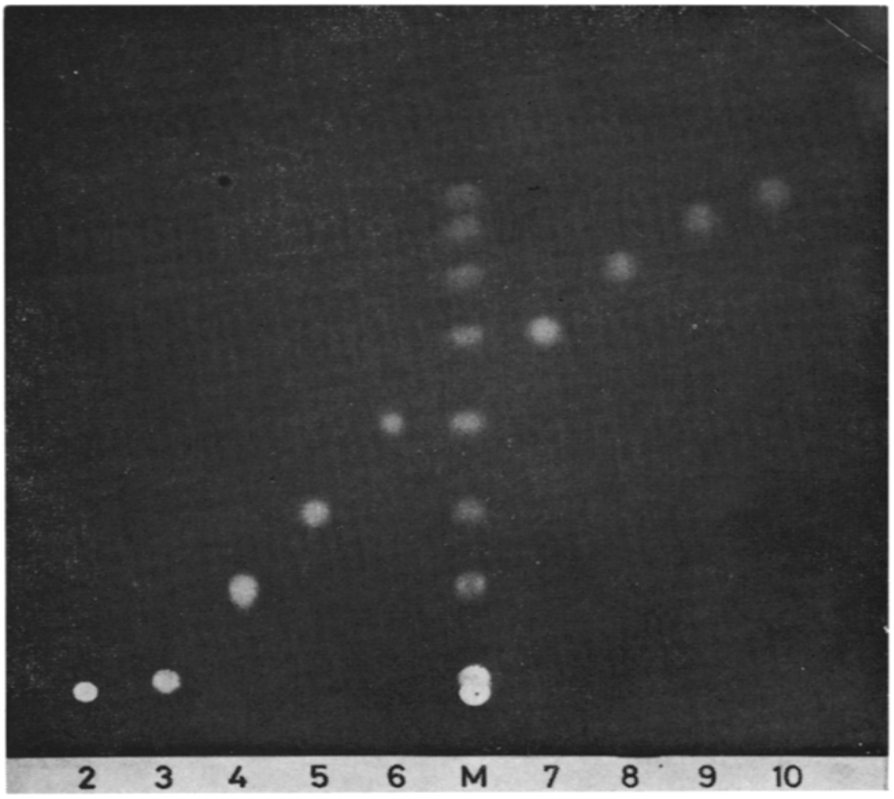

Fig. 1. Thin-layer chromatogram of aliphatic dibasic acids. The numerals and M indicate the number of carbon atoms of each acid and a mixture of all the test samples, respectively. Pre-treatment: 40% aqueous acetic acid. Temperature: 25°.

Monobasic acids such as C_6 or higher, present in the sample, did not interfere with the detection of the dibasic acids, because the monobasic acids migrated with the front.

Government Chemical Industrial Research Institute, Tokyo, Shibuya-ku, Tokyo (Japan)

SHOJI MIYAZAKI
YASUO SUHARA
TADASHI KOBAYASHI

1 D. BRAUN AND H. GEENEN, *J. Chromatog.*, 7 (1962) 56.
2 H. J. PETROWITZ AND G. PASTUSKA, *J. Chromatog.*, 7 (1962) 128.
3 N. S. RAJAGOPAL, P. K. SARASWATHY, M. R. SUBBARAM AND K. T. ACHAYA, *J. Chromatog.*, 24 (1966) 217.
4 E. BANCHER AND H. SCHERZ, *Mikrochim. Acta*, (1964) 1159.
5 E. KNAPPE AND D. PETERI, *Z. Anal. Chem.*, 188 (1962) 184; 190 (1962) 380.

Received August 28th, 1968

J. Chromatog., 39 (1969) 88–90

CHROM. 3807

Präzisierung der quantitativen Berberinbestimmung durch dünnschichtchromatographische Direktauswertung

Berberin ist als Hauptalkaloid mehrerer Berberis-Arten Bestandteil der daraus hergestellten und arzneilich verwendeten Verarbeitungsformen. Die bei der quantitativen Alkaloidbestimmung übliche Anreicherung durch alkalische Ätherausschüttelung führt zur Erfassung bestimmter Gruppen oder des Gesamtkomplexes der Berberisalkaloide. Durch Modellversuche an Berberinchloridlösung wurde beobachtet, dass mit Äther keine quantitative Ausschüttelung möglich ist. Darüber hinaus entstehen allein durch kurzzeitige Alkalisierung dünnschichtchromatographisch nachweisbare Sekundärprodukte. Das führte in jedem Fall zu Unterwerten mit Wiederfindungsraten von 84.0–85.8%.

Die DC–Fluorometrie gestattet die Direktmessung des Berberins bzw. seiner Salze (Fig. 1) ohne vorherige Anreicherung. Mit dem von KURONO *et al.*[1] beschriebenen Laufmittel (Butanol–Eisessig–Wasser, 7:1:2; Adsorbens: Kieselgel) lässt sich das Alkaloid in alkoholischen Berberis-Auszügen ausreichend von störenden Begleitstoffen abtrennen. Die dem Berberin zuzuordnende Fluoreszenz auf dem DC zeigte nach Alkalibehandlung der Auftraglösung gleiche Intensitätsminderungen wie das reine Alkaloid.

Zur quantitativen Bestimmung des Berberins werden von dem zerkleinerten Drogenmaterial (Sieb 5, DAB 7) 100 mg viermal mit je 15 ml Methanol (80%ig) 15 min am Rückfluss extrahiert. Die Abtrennung der Extraktionslösung erfolgt mittels G4-Glasfritte unter Nachwaschen. Die vereinigten Filtrate werden mit Methanol (80%ig) auf 100 ml ergänzt und zur Auftragung auf die DC-Platte mit dem gleichen Lösungsmittel so verdünnt, dass ein Gehalt von *ca.* 0.3 mg/100 ml vorliegt. Auf etwa gleiche Konzentrationen muss eingestellt werden, wenn die Bestimmung in Lösungen des Berberins bzw. in Berberisextrakten durchzuführen ist. Die Vergleichslösung