The separation and identification of some unusual coumarin derivatives by thin-layer chromatography on silica gel*

In view of an interest in coumarin derivatives as possible inhibitors of certain enzymic reactions in the human erythrocyte[1], it seemed necessary to have available a method by which these compounds could be separated and identified. Although paper chromatography[2,3] and thin layer chromatography[4,5] techniques have already been reported for the separation and identification of some coumarin derivatives, it was considered advantageous to develop methods utilizing the latter technique for a large number of these derivatives in various solvent systems.

The present report, therefore, describes methods which can be used to separate and identify a number of coumarin derivatives.

Experimental

Glass plates 20 cm by 20 cm were used. Distilled water (60 ml) was added to a flask containing 30 g of silica gel G and the flask was shaken vigorously for 90 sec. A layer 0.25 mm thick was applied to five glass plates using an applicator (Unoplan Leveler obtained from Shandon Scientific Company, Ltd, London N.W. 10, England). The plates stood for 10 min at room temperature, and were then heated in an air oven for 10 min at 110–112°; they were subsequently turned so that the final drying process (taking 50 min) occurred with the plates in the vertical position. The prepared plates were cooled and stored in a desiccator until used.

The coumarin derivatives, dissolved in acetone, were applied in 2 μg quantities 2 cm from the lower edge of the plate. Development was carried out in three separate systems: System I: acetone–2,2,4-trimethylpentane–water (100:40:1); System II: acetone–ethyl acetate–petroleum ether–water (100:100:33:3.5); System III: 4-methyl-2-pentanone–acetone–petroleum ether (60:20:20).

The petroleum ether (30–60°) was obtained from the J. T. Baker Chemical Company, the pentanone and pentane derivatives from Eastman.

The system utilized was placed in the bottom of a rectangular tank (Shandon Scientific Co., Ltd.) to a height of 1 cm. Two absorbent disposable wicks (20 × 25 cm) were placed on opposite inside walls of the tank and saturated with the developing solvent. After inserting the plate, the tank was sealed with two layers of masking tape. The plates were removed when the solvent system had ascended to a distance of approximately 3 cm from the upper edge of the plate.

Migration velocities for systems I, II and III were 30, 30 and 34 cm per hour. Compounds were detected by the use of a shortwave ultraviolet Minerallight Lamp (Ultra-Violet Products, Inc., San Gabriel, Calif.), which emitted at approximately 2537 Å. Those compounds which did not fluoresce were detected by a 1% solution of potassium permanganate. A yellow color on a violet background was observed after heating for 10 min at 110°. Melting points were determined with a Koffler micro melting point apparatus.

Results and discussion

Twelve of the compounds were coumarin derivatives, two were indanediones. The R_F

* Supported in part by Public Health Service Grants No. HD-00370 and HD-00361, National Institute of Child Health and Development.

TABLE I

THIN-LAYER CHROMATOGRAPHY OF SOME COUMARIN DERIVATIVES

Compound	*m.p.*	*I*		*II*		*III*		*Fluor.*	*$KMnO_4$*
		R_F	R_S	R_F	R_S	R_F	R_S		
Coumarin	67–68	0.80	1.0	0.90	1.0	0.73	1.0	−−	++
4-Hydroxycoumarin	212–213	0.26	0.33	0.31	0.34	0.12	0.16	++	++
7-Hydroxycoumarin	225–227	0.74	0.93	0.77	0.86	0.71	0.97	++	++
3,3′-Methylene-bis-(4-hydroxycoumarin)	289–292	0.28	0.35	0.46	0.51	0.15	0.21	++	++
3,3′-Thio-bis-(4-hydroxycoumarin)	292–294	0.17	0.21	0.32	0.36	0.12	0.16	+	+
3,3′-Methylene-bis-(4-hydroxy-7-methoxycoumarin)	270–273	0.24	0.30	0.37	0.41	0.14	0.19	+	−−
3,3′-Methylene-bis-(4-propionoxycoumarin)	243–245	0.80	1.0	0.85	0.94	0.81	1.11	+	−−
3,3′-Ethylidene-bis-(4-hydroxycoumarin)	171–173	0.44	0.55	0.63	0.70	0.44	0.60	++	++
β,β-Di-(4-hydroxy-3-coumarin)-ethyl chloride	255–258	0.15	0.19	0.16	0.18	0.05	0.07	++	+++
3,3′-Propylidene-bis-(4-hydroxycoumarin)	143–145	0.44	0.55	0.56	0.62	0.44	0.60	++	+
3,3′-Butylidene-bis-(4-hydroxycoumarin)	126–128	0.48	0.60	0.68	0.76	0.42	0.58	++	+
3,3′-Benzylidene-bis-(4-hydroxycoumarin)	230–232	0.44	0.55	0.66	0.73	0.54	0.74	++	−−
4-Hydroxycoumarin-3-carboxylic acid ethyl ester	139–140	0.14	0.18	0.15	0.17	0.04	0.06	+++	+++
2-Benzohydryl-1,3-indanedione	129–130	0.20	0.25	0.0	—	0.09	0.12	−−	+
1-(1,3-Diketo-2-indanyl)-1-phenyl-2-nitropropane	—	0.24	0.30	0.87	0.97	0.09	0.12	−−	+

and R_S values together with the magnitude of fluorescence or color development and melting points are shown in Table I. The R_S value is defined as the ratio of the distance moved from the origin by a derivative to the distance moved by coumarin itself.

Of the systems examined I and II were approximately equal in separating the coumarin derivatives. System III, on the other hand, had the greatest migration velocity and effected the separation of some derivatives which were not resolved with I and II.

The combination of fluorescence with ultraviolet light and potassium permanganate spray proved to be satisfactory for visualization of the coumarin derivatives. All the chromatographic spots were detected by one of the two methods and most compounds were detected by both.

Acknowledgements

The authors are grateful to Herschel Porter, Eli Lilly and Company, Indianapolis, Ind., for providing some of the compounds used.

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Received March 12th, 1964

J. Chromatog., 16 (1964) 229–231

Thin-film chromatography of some azo-dyestuffs

During a study of the metal complexes of heterocyclic azo-dyestuffs, it became necessary to check the purity of the azo-dyestuffs in order to ascertain whether a single product is formed on coupling the diazotate with the phenol. Thin-film chromatography has proved most successful for this purpose.

Thin-film plates were prepared in the usual way using Merck Silica Gel G, to which starch was added to aid binding properties. The plates were activated by drying in an oven at 110° for 20 min.

Many solvents were used to separate the dyestuffs, and those of composition listed below were most successful:

Solvent I: 50 ml 40–60° petroleum ether, 50 ml diethyl ether and 5 ml absolute ethanol.

Solvent II: 60 ml *n*-butanol, 20 ml absolute ethanol and 20 ml of 2 *N* aqueous ammonium hydroxide.